EXHIBIT A

PART 1 OF 2

US006175752B1

(12) United States Patent

Say et al.

(10) Patent No.: US 6,175,752 B1
(45) Date of Patent: Jan. 16, 2001

(54) **ANALYTE MONITORING DEVICE AND METHODS OF USE**

(75) Inventors: **James Say**, Alameda; **Michael F. Tomasco**, Cupertino, both of CA (US); **Adam Heller**, Austin, TX (US); **Yoram Gal**, Kibbutz Yagur (IL); **Behrad Aria**, Alameda, CA (US); **Ephraim Heller**, Oakland, CA (US); **Phillip John Plante**, Sunnyvale, CA (US); **Mark S. Vreeke**, Alameda, CA (US); **Keith A. Friedman**, Austin, TX (US); **Fredric C. Colman**, Berkeley, CA (US)

(73) Assignee: **Therasense, Inc.**, Alameda, CA (US)

(*) Notice: Under 35 U.S.C. 154(b), the term of this patent shall be extended for 0 days.

(21) Appl. No.: **09/070,677**

(22) Filed: **Apr. 30, 1998**

(51) **Int. Cl.**[7] **A61B 5/05**

(52) **U.S. Cl.** **600/345**; 600/365; 128/903

(58) **Field of Search** 600/300–301, 600/346, 306–309, 345–365, 372, 385–390; 604/174–180; 128/897–898, 903, 904, 920

(56) **References Cited**

U.S. PATENT DOCUMENTS

Re. 32,947	6/1989	Dormer et al. .
3,260,656	7/1966	Ross, Jr. .
3,653,841	4/1972	Klein .
3,719,564	3/1973	Lilly, Jr. et al. .
3,776,832	12/1973	Oswin et al. .
3,837,339	9/1974	Aisenberg et al. .
3,926,760	12/1975	Allen et al. .
3,972,320	8/1976	Kalman .
3,979,274	9/1976	Newman .
4,008,717	2/1977	Kowarski .
4,016,866	4/1977	Lawton .
4,055,175	10/1977	Clemens et al. .
4,059,406	11/1977	Fleet .
4,076,596	2/1978	Connery et al. .
4,098,574	7/1978	Dappen .
4,100,048	7/1978	Pompei et al. .

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

227 029 A3	9/1985	(DD) .
29 03 216	8/1979	(DE) .

(List continued on next page.)

OTHER PUBLICATIONS

Abruna, H. D. et al., "Rectifying Interfaces Using Two–Layer, Films of Electrochemically Polymerized Vinylpyridine and Vinylbipyridine Complexes of Ruthenium and Iron on Electrodes," *J. Am. Chem. Soc.*, 103(1):1–5 (Jan. 14, 1981).

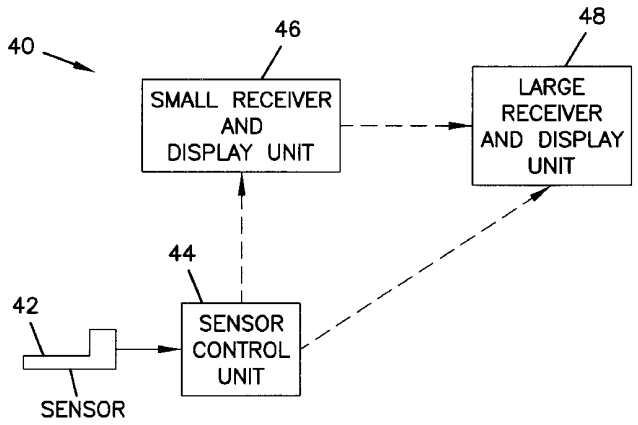
(List continued on next page.)

Primary Examiner—Cary O'Connor
Assistant Examiner—Michael Astorino
(74) *Attorney, Agent, or Firm*—Merchant & Gould P.C.

(57) **ABSTRACT**

An analyte monitor includes a sensor, a sensor control unit, and a display unit. The sensor has, for example, a substrate, a recessed channel formed in the substrate, and conductive material disposed in the recessed channel to form a working electrode. The sensor control unit typically has a housing adapted for placement on skin and is adapted to receive a portion of an electrochemical sensor. The sensor control unit also includes two or more conductive contacts disposed on the housing and configured for coupling to two or more contact pads on the sensor. A transmitter is disposed in the housing and coupled to the plurality of conductive contacts for transmitting data obtained using the sensor. The display unit has a receiver for receiving data transmitted by the transmitter of the sensor control unit and a display coupled to the receiver for displaying an indication of a level of an analyte. The analyte monitor may also be part of a drug delivery system to alter the level of the analyte based on the data obtained using the sensor.

94 Claims, 26 Drawing Sheets



U.S. PATENT DOCUMENTS

4,151,845	5/1979	Clemens .
4,168,205	9/1979	Danninger et al. .
4,172,770	10/1979	Semersky et al. .
4,178,916	12/1979	McNamara .
4,206,755	6/1980	Klein .
4,224,125	9/1980	Nakamura et al. .
4,240,438	12/1980	Updike et al. .
4,247,297	1/1981	Berti et al. .
4,340,458	7/1982	Lerner et al. .
4,352,960	10/1982	Dormer et al. .
4,356,074	10/1982	Johnson .
4,365,637	12/1982	Johnson .
4,366,033	12/1982	Richter et al. .
4,375,399	3/1983	Havas et al. .
4,384,586	5/1983	Christiansen .
4,390,621	6/1983	Bauer .
4,401,122	8/1983	Clark, Jr. .
4,404,066	9/1983	Johnson .
4,418,148	11/1983	Oberhardt .
4,427,770	1/1984	Chen et al. .
4,431,004	2/1984	Bessman et al. .
4,436,094	3/1984	Cerami .
4,440,175	4/1984	Wilkins .
4,450,842	5/1984	Zick et al. .
4,458,686	7/1984	Clark, Jr. .
4,461,691	7/1984	Frank .
4,469,110	9/1984	Slarna .
4,477,314	10/1984	Richter et al. .
4,484,987	11/1984	Gough .
4,522,690	6/1985	Venkatsetty .
4,524,114	6/1985	Samuels et al. .
4,526,661	7/1985	Steckhan et al. .
4,534,356	8/1985	Papadakis .
4,538,616	9/1985	Rogoff .
4,543,955	10/1985	Schroeppel .
4,545,382	10/1985	Higgins et al. .
4,552,840	11/1985	Riffer .
4,560,534	12/1985	Kung et al. .
4,571,292	2/1986	Liu et al. .
4,573,994	3/1986	Fischell et al. .
4,581,336	4/1986	Malloy et al. .
4,595,011	6/1986	Phillips .
4,619,754	10/1986	Niki et al. .
4,627,445	12/1986	Garcia et al. .
4,627,908	12/1986	Miller .
4,633,878	1/1987	Bombardieri .
4,637,403	1/1987	Garcia et al. .
4,650,547	3/1987	Gough .
4,654,197	3/1987	Lilja et al. .
4,655,880	4/1987	Liu .
4,655,885	4/1987	Hill et al. .
4,671,288	6/1987	Gough .
4,679,562	7/1987	Luksha .
4,680,268	7/1987	Clark, Jr. .
4,682,602	7/1987	Prohaska .
4,684,537	8/1987	Graetzel et al. .
4,685,463	8/1987	Williams .
4,703,756	11/1987	Gough et al. .
4,711,245	12/1987	Higgins et al. .
4,717,673	1/1988	Wrighton et al. .
4,721,601	1/1988	Wrighton et al. .
4,721,677	1/1988	Clark, Jr. .
4,726,378	2/1988	Kaplan .
4,726,716	2/1988	McGuire .
4,757,022	7/1988	Shults et al. .
4,758,323	7/1988	Davis et al. .
4,759,371	7/1988	Franetzki .
4,759,828	7/1988	Young et al. .
4,764,416	8/1988	Ueyama et al. .
4,776,944	10/1988	Janata et al. .
4,781,798	11/1988	Gough .
4,784,736	11/1988	Lonsdale et al. .
4,795,707	1/1989	Niiyama et al. .
4,796,634	1/1989	Huntsman et al. .
4,805,624	2/1989	Yao et al. .
4,813,424	3/1989	Wilkins .
4,815,469	3/1989	Cohen et al. .
4,820,399	4/1989	Senda et al. .
4,822,337	4/1989	Newhouse et al. .
4,830,959	5/1989	McNeil et al. .
4,832,797	5/1989	Vadgama et al. .
4,840,893	6/1989	Hill et al. .
4,848,351	7/1989	Finch .
4,871,351	10/1989	Feingold .
4,871,440	10/1989	Nagata et al. .
4,874,500	10/1989	Madou et al. .
4,890,620	1/1990	Gough .
4,894,137	1/1990	Takizawa et al. .
4,897,162	1/1990	Lewandowski et al. .
4,897,173	1/1990	Nankai et al. .
4,909,908	3/1990	Ross et al. .
4,911,794	3/1990	Parce et al. .
4,917,800	4/1990	Lonsdale et al. .
4,919,141	4/1990	Zier et al. .
4,919,767	4/1990	Vadgama et al. .
4,923,586	5/1990	Katayama et al. .
4,927,516	5/1990	Yamaguchi et al. .
4,934,369	6/1990	Maxwell .
4,935,105	6/1990	Churchouse .
4,935,345	6/1990	Guilbeau et al. .
4,938,860	7/1990	Wogoman .
4,944,299	7/1990	Silvian .
4,950,378	8/1990	Nagara .
4,953,552 *	9/1990	DeMarzo 600/300
4,954,129	9/1990	Giuliani et al. .
4,969,468	11/1990	Byers et al. .
4,970,145	11/1990	Bennetto et al. .
4,974,929	12/1990	Curry .
4,986,271	1/1991	Wilkins .
4,994,167	2/1991	Shults et al. .
5,001,054	3/1991	Wagner .
5,050,612	9/1991	Matsumura .
5,058,592	10/1991	Whisler .
5,070,535	12/1991	Hochmair et al. .
5,082,550	1/1992	Rishpon et al. .
5,082,786	1/1992	Nakamoto .
5,089,112	2/1992	Skotheim et al. .
5,095,904	3/1992	Seligman et al. .
5,101,814	4/1992	Palti .
5,108,564	4/1992	Szuminsky et al. .
5,109,850	5/1992	Blanco et al. .
5,120,420	6/1992	Nankai et al. .
5,126,034	6/1992	Carter et al. .
5,133,856	7/1992	Yamaguchi et al. .
5,135,003	8/1992	Souma .
5,141,868	8/1992	Shanks et al. .
5,161,532	11/1992	Joseph .
5,165,407	11/1992	Wilson et al. .
5,174,291	12/1992	Schoonen et al. .
5,190,041	3/1993	Palti .
5,192,416	3/1993	Wang et al. .
5,198,367	3/1993	Aizawa et al. .
5,202,261	4/1993	Musho et al. .
5,205,920	4/1993	Oyama et al. .
5,208,154	5/1993	Weaver et al. .
5,209,229	5/1993	Gilli .
5,217,595	6/1993	Smith et al. .
5,229,282	7/1993	Yoshioka et al. .
5,250,439	10/1993	Musho et al. .
5,262,035	11/1993	Gregg et al. .
5,262,305	11/1993	Heller et al. .

5,264,103		11/1993	Yoshioka et al. .	
5,264,104		11/1993	Gregg et al. .	
5,264,106		11/1993	McAleer et al. .	
5,271,815		12/1993	Wong .	
5,279,294		1/1994	Anderson et al. .	
5,286,362		2/1994	Hoenes et al. .	
5,286,364		2/1994	Yacynych et al. .	
5,288,636		2/1994	Pollmann et al. .	
5,293,546		3/1994	Tadros et al. .	
5,320,098		6/1994	Davidson .	
5,320,725		6/1994	Gregg et al. .	
5,322,063	*	6/1994	Allen et al.	600/372
5,337,747		8/1994	Neftel .	
5,352,348		10/1994	Young et al. .	
5,356,348		10/1994	Young et al. .	
5,356,786		10/1994	Heller et al. .	
5,368,028		11/1994	Palti .	
5,372,133		12/1994	Hogen Esch .	
5,376,251		12/1994	Kaneko et al. .	
5,378,628		1/1995	Gratzel et al. .	
5,387,327		2/1995	Khan .	
5,390,671		2/1995	Lord et al. .	
5,391,250		2/1995	Cheney, II et al. .	
5,395,504		3/1995	Saurer et al. .	
5,400,782	*	3/1995	Beaubiah	600/309
5,411,647		5/1995	Johnson et al. .	
5,437,999		8/1995	Diebold et al. .	
5,469,846		11/1995	Khan .	
5,491,474	*	2/1996	Suni et al.	128/903
5,494,562		2/1996	Maley et al. .	
5,496,453		3/1996	Uenoyama et al. .	
5,497,772		3/1996	Schulman et al. .	
5,531,878		7/1996	Vadgama et al. .	
5,545,191		8/1996	Mann et al. .	
5,560,357		10/1996	Faupel et al. .	
5,562,713	*	10/1996	Silvian	128/903
5,565,085		10/1996	Ikeda et al. .	
5,567,302		10/1996	Song et al. .	
5,568,806		10/1996	Cheney, II et al. .	
5,569,186		10/1996	Lord et al. .	
5,582,184		12/1996	Erickson et al. .	
5,582,697		12/1996	Ikeda et al. .	
5,582,698		12/1996	Flaherty et al. .	
5,586,553		12/1996	Halili et al. .	
5,589,326		12/1996	Deng et al. .	
5,593,852		1/1997	Heller et al. .	
5,596,150		1/1997	Arndt et al. .	
5,617,851		4/1997	Lipkovker .	
5,628,890		5/1997	Carter et al. .	
5,651,869		7/1997	Yoshioka et al. .	
5,660,163		8/1997	Schulman et al. .	
5,670,031		9/1997	Hintsche et al. .	
5,680,858		10/1997	Hansen et al. .	
5,682,233		10/1997	Brinda .	
5,695,623		12/1997	Michel et al. .	
5,708,247		1/1998	McAleer et al. .	
5,711,297	*	1/1998	Iliff et al.	600/300
5,711,861	*	1/1998	Ward et al.	600/365
5,711,862		1/1998	Sakoda et al. .	
5,741,211		4/1998	Renirie et al. .	
5,771,001	*	6/1998	Cobb	128/903
5,791,344	*	8/1998	Schulman et al.	600/300
5,800,420	*	9/1998	Gross et al.	604/890.1
5,807,375		9/1998	Gross et al. .	
5,820,551	*	10/1998	Hill et al.	600/365
5,820,622	*	10/1998	Gross et al.	604/890.1
5,822,715		10/1998	Worthington et al. .	
5,827,184	*	10/1998	Netherly et al.	600/385
5,840,020		11/1998	Heinonen et al. .	
5,842,983	*	12/1998	Abel et al.	600/372
5,885,211	*	3/1999	Eppstein et al.	600/365
5,954,685	*	9/1999	Tierny	600/386
5,971,922	*	10/1999	Arita et al.	600/365

FOREIGN PATENT DOCUMENTS

3934299	10/1990	(DE) .
0 010 375 A1	4/1980	(EP) .
0 026 995 A1	4/1981	(EP) .
0 048 090 A2	3/1982	(EP) .
0 078 636 A1	5/1983	(EP) .
0 096 228 A1	12/1983	(EP) .
0 125 139 A2	11/1984	(EP) .
0 127 958 A2	12/1984	(EP) .
0 136 362 A1	4/1985	(EP) .
0 170 375 A2	2/1986	(EP) .
0 177 743 A2	4/1986	(EP) .
0 080 304 B1	5/1986	(EP) .
0 184 909 A2	6/1986	(EP) .
0 206 218 A2	12/1986	(EP) .
0 230 472 A1	8/1987	(EP) .
0 241 309 A3	10/1987	(EP) .
0 245 073 A2	11/1987	(EP) .
0 278 647 A2	8/1988	(EP) .
0 359 831 A1	3/1990	(EP) .
0 368 290 A1	10/1990	(EP) .
390 390 A1	10/1990	(EP) .
0 400 918 A1	12/1990	(EP) .
0 453 283 A1	10/1991	(EP) .
0 470 290 A1	2/1992	(EP) .
0 127 958 B2	3/1992	(EP) .
0 255 291 B1	6/1992	(EP) .
1394171	5/1975	(GB) .
1599241	9/1981	(GB) .
2 073 891	10/1981	(GB) .
2 154 003	2/1988	(GB) .
2 204 408	11/1988	(GB) .
2 254 436	10/1992	(GB) .
54-41191	4/1979	(JP) .
55-10581	1/1980	(JP) .
55-10583	1/1980	(JP) .
55-10584	1/1980	(JP) .
55-12406	1/1980	(JP) .
56-163447	12/1981	(JP) .
57-70448	4/1982	(JP) .
60-173457	9/1985	(JP) .
60-173458	9/1985	(JP) .
60-173459	9/1985	(JP) .
61-90050	5/1986	(JP) .
62-85855	4/1987	(JP) .
62-114747	5/1987	(JP) .
63-58149	3/1988	(JP) .
63-128252	5/1988	(JP) .
63-139246	6/1988	(JP) .
63-294799	12/1988	(JP) .
63-317757	12/1988	(JP) .
63-317758	12/1988	(JP) .
1-114746	5/1989	(JP) .
1-114747	5/1989	(JP) .
1-124060	5/1989	(JP) .
1-134244	5/1989	(JP) .
1-156658	6/1989	(JP) .
2-62958	3/1990	(JP) .
2-120655	5/1990	(JP) .
2-287145	11/1990	(JP) .
2-310457	12/1990	(JP) .
3-26956	2/1991	(JP) .
3-28752	2/1991	(JP) .
3-202764	9/1991	(JP) .
5-72171	3/1993	(JP) .
5-196595	8/1993	(JP) .
6-190050	7/1994	(JP) .
7-55757	3/1995	(JP) .

7-72585	3/1995	(JP) .
8-285814	11/1996	(JP) .
8-285815	11/1996	(JP) .
9-21778	1/1997	(JP) .
9-101280	4/1997	(JP) .
9-285459	11/1997	(JP) .
10-170471	6/1998	(JP) .
1281988 A1	1/1987	(SU) .
WO 89/05119	11/1985	(WO) .
WO 89/08713	9/1989	(WO) .
WO 90/05300	5/1990	(WO) .
WO 90/05910	5/1990	(WO) .
WO 91/01680	2/1991	(WO) .
WO 91/04704	4/1991	(WO) .
WO 91/15993	10/1991	(WO) .
WO 92/13271	8/1992	(WO) .
WO 94/20602	9/1994	(WO) .
WO 94/27140	11/1994	(WO) .
WO 96/30431	10/1996	(WO) .
WO 96/35370	11/1996	(WO) .
WO 97/02847	1/1997	(WO) .
WO 97/19344	5/1997	(WO) .
WO 97/42882	11/1997	(WO) .
WO 97/42883	11/1997	(WO) .
WO 97/42886	11/1997	(WO) .
WO 97/42888	11/1997	(WO) .
WO 97/43962	11/1997	(WO) .

OTHER PUBLICATIONS

Albery, W. J. et al., "Amperometric enzyme electrodes. Part II. Conducting salts as electrode materials for the oxidation of glucose oxidase," *J. Electroanal. Chem. Interfacial Electrochem.,* 194(2) (1 page—Abstract only) (1985).

Albery, W. J. et al., "Amperometric Enzyme Electrodes," *Phil. Trans. R Soc. Lond.* B316:107–119 (1987).

Alcock, S. J. et al., "Continuous Analyte Monitoring to Aid Clinical Practice," *IEEE Engineering in Medicine and Biology,* 319–325 (1994).

Anderson, L. B. et al., "Thin–Layer Electrochemistry: Steady–State Methods of Studying Rate Processes," *J. Electroanal. Chem.,* 10:295–395 (1965).

Bartlett, P. N. et al., "Covalent Binding of Electron Relays to Glucose Oxidation," *J. Chem. Soc. Chem. Commun.,* 1603–1604 (1987).

Bartlett, P. N. et al., "Modification of glucose oxidase by tetrathiafulvalene," *J. Chem. Soc., Chem. Commun.,* 16 (1 page—Abstract only) (1990).

Bartlett, P. N. et al., "Strategies for the Development of Amperometric Enzyme Electrodes," *Biosensors,* 3:359–379 (1987/88).

Bindra, D.S. et al., "Design and in Vitro Studies of a Needle–Type Glucose Sensor for Subcutaneous Monitoring", *Anal. Chem.,* 63(17):1692–1696 (Sep. 1, 1991).

Bobbioni–Harsch, E. et al., "Lifespan of subcutaneous glucose sensors and their performances during dynamic glycaemia changes in rats," *J. Biomed. Eng.* 15:457–463 (1993).

Brandt J. et al., "Covalent attachment of proteins to polysaccharide carriers by means of benzoquinone," *Biochim. Biophys. Acta,* 386(1) (1 page Abstract only) (1975).

Brownlee, M. et al., "A Glucose–Controlled Insulin–Delivery System: Semisynthetic Insulin Bound to Lectin", *Science,* 206(4423):1190–1191 (Dec. 7, 1979).

Cass, A.E.G. et al., "Ferricinum Ion As An Electron Acceptor for Oxido–Reductases," *J. Electroanal. Chem.,* 190:117–127 (1985).

Cass, A.E.G. et al., "Ferrocene–Mediated Enzyme Electrode for Amperometric Determination of Glucose", *Anal. Chem.,* 56(4):667–671 (Apr. 1984).

Castner, J. F. et al., "Mass Transport and Reaction Kinetic Parameters Determined Electrochemically for Immobilized Glucose Oxidase," *Biochemistry,* 23(10):2203–2210 (1984).

Claremont, D.J. et al., "Biosensors for Continuous In Vivo Glucose Monitoring", *IEEE Engineering in Medicine and Biology Society 10th Annual International Conference,* New Orleans, Louisiana, 3 pgs. (Nov. 4–7, 1988).

Clark L.C. et al., "Differential Anodic Enzyme Polarography for the Measurement of Glucose", *Oxygen Transport to Tissue: Instrumentation, Methods, and Physiology,* 127–132 (1973).

Clark, L.C., Jr. et al., "Electrode Systems for Continuous Monitoring in Cardiovascular Surgery," *Annals New York Academy of Sciences,* pp. 29–45 (1962).

Clark, L.C. et al., "Long–term Stability of Electroenzymatic Glucose Sensors Implanted in Mice," *Trans. Am. Soc. Artif. Intern. Organs,* XXXIV:259–265 (1988).

Clarke, W. L., et al., "Evaluating Clinical Accuracy of Systems for Self–Monitoring of Blood Glucose," *Diabetes Care,* 10(5):622–628 (Sep.–Oct. 1987).

Csöregi, E. et al., "Design, Characterization, and One–Point in Vivo Calibration of a Subcutaneously Implanted Glucose Electrode," *Anal. Chem.* 66(19):3131–3138 (Oct. 1, 1994).

Csöregi, E. et al., "Design and Optimization of a Selective Subcutaneously Implantable Glucose Electrode Based on "Wired" Glucose Oxidase," *Anal. Chem.* 67(7):1240–1244 (Apr. 1, 1995).

Csöregi, E. et al., "On–Line Glucose Monitoring by Using Microdialysis Sampling and Amperometric Detection Based on "Wired" Glucose Oxidase in Carbon Paste," *Mikrochim. Acta.* 121:31–40 (1995).

Davis, G., "Electrochemical Techniques for the Development of Amperometric Biosensors", *Biosensors,* 1:161–178 (1985).

Degani, Y. et al., "Direct Electrical Communication between Chemically Modified Enzymes and Metal Electrodes. 1. Electron Transfer from Glucose Oxidase to Metal Electrodes via Electron Relays, Bound Covalently to the Enzyme," *J. Phys. Chem.* 91(6):1285–1289 (1987).

Degani, Y. et al., "Direct Electrical Communication between Chemically Modified Enzymes and Metal Electrodes. 2. Methods for Bonding Electron–Transfer Relays to Glucose Oxidase and D–Amino–Acid Oxidase," *J. Am. Chem. Soc.,* 110(8):2615–2620 (1988).

Degani, Y. et al., "Electrical Communication between Redox Centers of Glucose Oxidase and Electrodes via Electrostatically and Covalently Bound Redox Polymers," *J. Am. Chem. Soc.,* 111:2357–2358 (1989).

Denisevich, P. et al., "Unidirectional Current Flow and Charge State Trapping at Redox Polymer Interfaces on Bilayer Electrodes: Principles, Experimental Demonstration, and Theory," *J. Am. Chem. Soc.,* 103(16):4727–4737 (1981).

Dicks, J. M., "Ferrocene modified polypyrrole with immobilised glucose oxidase and its application in amperometric glucose microbiosensors," *Ann. Biol. clin.,* 47:607–619 (1989).

Engstrom, R.C., "Electrochemical Pretreatment of Glassy Carbon Electrodes", *Anal. Chem.,* 54(13):2310–2314 (Nov. 1982).

Engstrom, R.C. et al., "Characterization of Electrochemically Pretreated Glassy Carbon Electrodes", *Anal. Chem.,* 56(2):136–141 (Feb. 1984).

Ellis, C. D., "Selectivity and Directed Charge Transfer through an Electroactive Metallopolymer Film," *J. Am. Chem. Soc.,* 103(25):7480–7483 (1981).

Feldman, B.J. et al., "Electron Transfer Kinetics at Redox Polymer/Solution Interfaces Using Microelectrodes and Twin Electrode Thin Layer Cells", *J. Electroanal. Chem.,* 194(1):63–81 (Oct. 10, 1985).

Fischer, H. et al., "Intramolecular Electron Transfer Mediated by 4,4'–Bipyridine and Related Bridging Groups", *J. Am. Chem. Soc.,* 98(18):5512–5517 (Sep. 1, 1976).

Foulds, N.C. et al., "Enzyme Entrapment in Electrically Conducting Polymers," *J. Chem. Soc., Faraday Trans 1.,* 82:1259–1264 (1986).

Foulds, N.C. et al., "Immobilization of Glucose Oxidase in Ferrocene–Modified Pyrrole Polymers," *Anal. Chem.,* 60(22):2473–2478 (Nov. 15, 1988).

Frew, J.E. et al., "Electron–Transfer Biosensors", *Phil. Trans. R Soc. Lond.,* B316:95–106 (1987).

Gorton, L. et al., "Selective detection in flow analysis based on the combination of immobilized enzymes and chemically modified electrodes," *Analytica Chimica Acta.,* 250:203–248 (1991).

Gregg, B. A. et al., "Cross–Linked Redox Gels Containing Glucose Oxidase for Amperometric Biosensor Applications," *Analytical Chemistry,* 62(3):258–263 (Feb. 1, 1990).

Gregg, B. A. et al., "Redox Polymer Films Containing Enzymes. 1. A Redox–Conducting Epoxy Cement: Synthesis, Characterization, and Electrocatalytic Oxidation of Hydroquinone," *J. Phys. Chem.,* 95(15):5970–5975 (1991).

Hale, P D et al., "A New Class of Amperometric Biosensor Incorporating a Polymeric Electron–Transfer Mediator," *J. Am. Chem. Soc.,* 111(9):3482–3484 (1989).

Harrison, D.J. et al., "Characterization of Perfluorosulfonic Acid Polymer Coated Enzyme Electrodes and a Miniaturized Integrated Potentiostat for Glucose Analysis in Whole Blood", *Anal. Chem.,* 60(19):2002–2007 (Oct. 1, 1988).

Hawkridge, F. M. et al., "Indirect Coulometric Titration of Biological Electron Transport Components," *Analytical Chemistry,* 45(7):1021–1027 (Jun. 1973).

Heller, A., "Amperometric biosensors based on three–dimensional hydrogel–forming epoxy networks," *Sensors and Actuators B.* 13–14:180–183 (1993).

Heller, A., "Electrical Connection of Enzyme Redox Centers to Electrodes," *J. Phys. Chem.,* 96(9):3579–3587 (1992).

Heller, A., "Electrical Wiring of Redox Enzymes," *Acc. Chem. Res.,* 23(5):129–134 (1990).

Ianniello, R.M. et al. "Immobilized Enzyme Chemically Modified Electrode as an Amperometric Sensor", *Anal. Chem.,* 53(13):2090–2095 (Nov. 1981).

Ianniello, R.M. et al., "Differential Pulse Voltammetric Study of Direct Electron Transfer in Glucose Oxidase Chemically Modified Graphite Electrodes", *Anal. Chem.,* 54(7):1098–1101 (Jun. 1981).

Ikeda, T. et al., "Glucose oxidase–immobilized benzoquinone–carbon paste electrode as a glucose sensor," *Agric. Biol. Chem.,* 49(2) (1 page—Abstract only) (1985).

Ikeda, T. et al., "Kinetics of Outer–Sphere Electron Transfers Between Metal Complexes in Solutions and Polymeric Films on Modified Electrodes", *J. Am. Chem. Soc.,* 103(25):7422–7425 (Dec. 16, 1981).

Johnson, J. M. et al., "Potential–Dependent Enzymatic Activity in an Enzyme Thin–Layer Cell," *Anal. Chem.* 54:1377–1383 (1982).

Johnson, K. W., "Reproducible Electrodeposition of Biomolecules for the Fabrication of Miniature Electroenzymatic Biosensors", *Sensors and Actuators B Chemical,* B5:85–89 (1991).

Jonsson, G. et al., "An Amperometric Glucose Sensor Made by Modification of a Graphite Electrode Surface With Immobilized Glucose Oxidase and Adsorbed Mediator", *Biosensors,* 1:355–368 (1985).

Josowicz, M. et al., "Electrochemical Pretreatment of Thin Film Platinum Electrodes", *J. Elecrochem. Soc.,* 135(1):112–115 (Jan. 1988).

Katakis, I. et al., "Electrostatic Control of the Electron Transfer Enabling Binding of Recombinant Glucose Oxidase and Redox Polyelectrolytes," *J. Am. Chem. Soc.,* 116(8):3617–3618 (1994).

Katakis, I. et al., "L–α–Glycerophosphate and L–Lactate Electrodes Based on the Electrochemical "Wiring" of Oxidases," *Analytical Chemistry,* 64(9):1008–1013 (May 1, 1992).

Kenausis, G. et al., "Wiring of glucose oxidase and lactate oxidase within a hydrogel made with poly(vinyl pyridine) complexed with $[Os(4,4'–dimethoxy–2,2'–bipyridine)_2C1]^{+/2+}$," *J. Chem. Soc., Faraday Trans.,* 90(20):4131–4136 (1996).

Koudelka, M. et al., "In–Vivo Behaviour of Hypodermically Implanted Microfabricated Glucose Sensors", *Biosensors & Bioelectronics,* 6(1):31–36 (1991).

Kulys, J. et al., "Mediatorless peroxidase electrode and preparation of bienzyme sensors," *Bioelectrochemistry and Bioenergetics,* 24:305–311 (1990).

Lager, W. et al., "Implantable Electrocatalytic Glucose Sensor," *Horm. Metab. Res.,* 26:526–530 (Nov. 1994).

Lindner, E. et al. "Flexible (Kapton–Based) Microsensor Arrays of High Stability for Cardiovascular Applications", *J. Chem. Soc, Faraday Trans.,* 89(2):361–367 (Jan. 21, 1993).

Maidan, R. et al., "Elimination of Electrooxidizable Interferant–Produced Currents in Amperometric Biosensors," *Analytical Chemistry,* 64(23):2889–2896 (Dec. 1, 1992).

Mastrototaro, J.J. et al., "An Electroenzymatic Glucose Sensor Fabricated on a Flexible Substrate", *Sensors and Biosensors B Chemical,* B5:139–144 (1991).

McNeil, C. J. et al., "Thermostable Reduced Nicotinamide Adenine Dinucleotide Oxidase: Application to Amperometric Enzyme Assay," *Anal. Chem.,* 61(1):25–29 (Jan. 1, 1989).

Miyawaki, O. et al., "Electrochemical and Glucose Oxidase Coenzyme Activity of Flavin Adenine Dinucleotide Covalently Attached to Glassy Carbon at the Adenine Amino Group", *Biochimica et Biophysica Acta,* 838:60–68 (1985).

Moatti–Sirat, D. et al., "Evaluating in vitro and in vivo the inteference of ascorbate and acetaminophen on glucose detection by a needle–type glucose sensor," *Biosensors & Bioelectronics,* 7(5):345–352 (1992).

Moatti–Sirat, D. et al., "Reduction of acetaminophen interference in glucose sensors by a composite Nafion membrane: demonstration in rats and man," *Diabetologia,* 37(6) (1 page—Abstract only) (Jun. 1994).

Moatti–Sirat, D. et al., "Towards continuous glucose monitoring: in vivo evaluation of a miniaturized glucose sensor implanted for several days in rat subcutaneous tissue," *Diabetologia,* 35(3) (1 page—Abstract only) (Mar. 1992).

Nagy, G. et al., "A New Type of Enzyme Electrode: The Ascorbic Acid Eliminator Electrode," *Life Sciences,* 31(23):2611–2616 (1982).

Nakamura, S. et al., "Effect of Periodate Oxidation on the Structure and Properties of Glucose Oxidase," *Biochimica et Biophysica Acta.,* 445:294–308 (1976).

Narazimhan, K. et al., "p–Benzoquinone activation of metal oxide electrodes for attachment of enzymes," *Enzyme Microb. Technol.,* 7(6) (1 page—Abstract only) (1985).

Ohara, T. J. et al., "Glucose Electrodes Based on Cross–Linked $[Os(bpy)_2C1]^{+/2+}$ Complexed Poly(1–vinylimadazole) Films," *Analytical Chemistry,* 65(23):3512–3516 (Dec. 1, 1993).

Ohara, T. J., "Osmium Bipyridyl Redox Polymers Used in Enzyme Electrodes," *Platinum Metals Rev.,* 39(2):54–62 (Apr. 1995).

Ohara, T. J. et al., ""'Wired" Enzyme Electrodes for Amperometric Determination of Glucose or Lactate in the Presence of Interfering Substances," *Analytical Chemistry,* 66(15):2451–2457 (Aug. 1, 1994).

Olievier, C. N. et al., "In vivo Measurement of Carbon Dioxide Tension with a Miniature Electrode," *Pflugers Arch.* 373:269–272 (1978).

Paddock, R. et al., "Electrocatalytic reduction of hydrogen peroxide via direct electron transfer from pyrolytic graphite electrodes to irreversibly adsorbed cytochrome c peroxidase," *J. Electroanal. Chem.,* 260:487–494 (1989).

Palleschi, G. et al., "A Study of Interferences in Glucose Measurements in Blood by Hydrogen Peroxide Based Glucose Probes", *Anal. Biochem.,* 159:114–121 (1986).

Pankramov, I. et al., "Sol–gel derived renewable–surface biosensors," *Journal of Electroanalytical Chemistry,* 393:35–41 (1995).

Pathak, C. P. et al., "Rapid Photopolymerization of Immunoprotective Gels in Contact with Cells and Tissue," *J. Am. Chem. Soc.,* 114(21):8311–8312 (1992).

Pickup, J., "Developing glucose sensors for in vivo use," *Tibtech,* 11:285–289 (Jul. 1993).

Pickup, J. C. et al., "In vivo molecular sensing in diabetes mellitus: an implantable glucose sensor with direct electron transfer," *Diabetologia,* 32(3):213–217 (1989).

Pickup, J. et al., "Potentially–implantable, amperometric glucose sensors with mediated electron transfer: improving the operating stability," *Biosensors,* 4(2) (1 page—Abstract only) (1989).

Pishko, M.V. et al., "Amperometric Glucose Microelectrodes Prepared Through Immobilization of Glucose Oxidase in Redox Hydrogels", *Anal. Chem.,* 63(20):2268–2272 (Oct. 15, 1991).

Poitout, V. et al., "A glucose monitoring system for on line estimation in man of blood glucose concentration using a miniaturized glucose sensor implanted in the subcutaneous tissue and a wearable control unit," *Diabetologia,* 36(7) (1 page—Abstract only) (Jul. 1993).

Poitout, V. et al., "Calibration in dogs of a subcutaneous miniaturized glucose sensor using a glucose meter for blood glucose determination," *Biosensors & Bioelectronics,* 7:587–592 (1992).

Poitout, V. et al., "In vitro and in vivo evaluation in dogs of a miniaturized glucose sensor," *ASAIO Transactions,* 37(3) (1 page—Abstract only) (Jul.–Sep. 1991).

Pollak, A. et al., "Enzyme immobilization by Condensation Copolymerization into Cross–Linked Polyacrylamide Gels," *J. Am. Chem. Soc.,* 102(20):6324–6336 (1980).

Reach, G. et al., "Can Continuous Glucose Monitoring Be Used for the Treatment of Diabetes?" *Analytical Chemistry,* 64(6):381–386 (Mar. 15, 1992).

Rebrin, K. et al., "Automated Feedback Control of subcutaneous Glucose Concentration in Diabetic Dogs", *Diabetologia,* 32(8):573–576 (Aug. 1989).

Sakakida, M. et al., "Ferrocene–mediate needle–type glucose sensor covered with newly designed biocompatible membrane," *Sensors and Actuators B,* 13–14:319–322 (1993).

Samuels, G. J. et al., "An Electrode–Supported Oxidation Catalyst Based on Ruthenium (IV). pH "Encapsulation" in a Polymer Film," *J. Am. Chem. Soc.,* 103(2):307–312 (1981).

Sasso, S.V. et al., "Electropolymerized 1,2–Diaminobenzene as a Means to Prevent Interferences and Fouling and to Stabilize Immobilized Enzyme in Electrochemical Biosensors", *Anal. Chem.,* 62(11):1111–1117 (Jun. 1, 1990).

Scheller, F. et al., "Enzyme electrodes and their application," *Phil. Trans. R Soc. Lond.,* B 316:85–94 (1987).

Schmehl, R.H. et al., "The Effect of Redox Site Concentration on the Rate of Mediated Oxidation of Solution Substrates by a Redox Copolymer Film", *J. Electroanal. Chem.,* 152:97–109 (Aug. 25, 1983).

Shichiri, M. et al., "Glycaemic Control in Pancreatomized Dogs with a Wearable Artificial Endocrine Pancreas", *Diabetologia,* 24(3):179–184 (Mar. 1983).

Sirtampalam, G. et al., "Surface–Modified Electrochemical Detector for Liquid Chromatography", *Anal. Chem.,* 55(9):1608–1610 (Aug. 1983).

Soegijoko, S. et al., *Horm. Metabl. Res., Suppl. Ser.* 12 (1 page—Abstract only) (1982).

Sprules, S. D. et al., "Evaluation of a New Disposable Screen–Printed Sensor Strip for the Measurement of NADH and Its Modification to Produce a Lactate Biosensor Employing Microliter Volumes," *Electroanalysis,* 8(6):539–543 (1996).

Sternberg, F. et al., "Calibration Problems of Subcutaneous Glucosensors when Applied "In–Situ" in Man," *Horm. metabl. Res.* 26:524–525 (1994).

Sternberg, R. et al., "Covalent Enzyme Coupling on Cellulose Acetate Membranes for Glucose Sensor Development," *Analytical Chemistry,* 60(24):2781–2786 (Dec. 15, 1988).

Sternberg, R. et al., "Study and Development of Multilayer Needle–type Enzyme–based Glucose Microsensors," *Biosensors,* 4:27–40 (1988).

Suckane, M., "Immobilization of glucose isomerase," *Zeitschrift fur Allgemeine Mikrobiologie,* 22(8):565–576 (1982).

Tajima, S. et al., "Simultaneous Determination of Glucose and 1,5–Anydroglucitol", *Chemical Abstracts,* 111(25):394 111:228556g (Dec. 18, 1989).

Tarasevich, M.R. "Bioelectrocatalysis", *Comprehensive Treasige of Electrochemistry,* 10 (Ch. 4):231–295 (1985).

Tatsuma, T. et al., "Enzyme Monolayer– and Bilayer–Modified Tin Oxide Electrodes for the Determination of Hydrogen Peroxide and Glucose," *Anal. Chem.,* 61(21):2352–2355 (Nov. 1, 1989).

Taylor, C. et al., "'Wiring' of glucose oxidase within a hydrogel made with polyvinyl imidazole complexed with $[(Os–4,4'–dimethoxy–2,2'–bipyridine)C1]^{+/2+}$," *Journal of Electroanalytical Chemistry,* 396:511–515 (1995).

Trojanowicz, M. et al., "Enzyme Entrapped Polypyrrole Modified Electrode for Flow–Injection Determination of Glucose," *Biosensors & Bioelectronics,* 5:149–156 (1990).
Turner, A.P.F. et al., "Diabetes Mellitus: Biosensors for Research and Management", *Biosensors,* 1:85–115 (1985).
Turner, R.F.B. et al., "A Biocompatible Enzyme Electrode for Continuous in vivo Glucose Monitoring in Whole Blood," *Sensors and Actuators,* B1(1–6):561–564 (Jan. 1990).
Tuzhi, P. et al., "Constant Potential Pretreatment of Carbon Fiber Electrodes for In vivo Electrochemistry", *Analytical Letters,* 24(6):935–945 (1991).
Umaha, M., "Protein–Modified Electrochemically Active Biomaterial Surface," *U.S. Army Research Office Report,* (12 pages) (Dec. 1988).
Urban, G. et al., "Miniaturized Thin–Film Biosensors Using Covalently Immobilized Glucose Oxidase", *Biosensors & Bioelectronics,* 6(7):555–562 (1991).
Velho, G. et al., "In Vitro and In Vivo Stability of Electrode Potentials in Needle–Type Glucose Sensors", *Diabetes,* 38(2):164–171 (Feb. 1989).
Velho, G. et al., "Strategies for calibrating a subcutaneous glucose sensor," *Biomed. Biochim. Acta,* 48(11/12);957–964 (1989).
Von Woedtke, T. et al., "In Situ Calibration of Implanted Electrochemical Glucose Sensors," *Biomed. Biochim. Acta,* 48(11/12):943–952 (1989).
Vrecke, M. S. et al., "Chapter 15: Hydrogen Peroxide Electrodes Based on Electrical Connection of Redox Centers of Various Peroxidases to Electrodes through a Three–Dimensional Electron–Relaying Polymer Network," *Diagnostic Biosensor Polymers,* 7 pgs. (Jul. 26, 1993).
Vrecke, M. et al., "Hydrogen Peroxide and β–Nicotinamide Adenine Dinucleotide Sensing Amperometric Electrodes Based on Electrical Connection of Horseradish Peroxidase Redox Centers to Electrodes through a Three–Dimensional Electron Relaying Polymer Network," *Analytical Chemistry,* 64(24):3084–3090 (Dec. 15, 1992).
Wang, J. et al., "Activation of Glassy Carbon Electrodes by Alternating Current Electrochemical Treatment", *Analytica Chimica Acta,* 167:325–334 (Jan. 1985).
Wang, J. et al., "Amperometric biosensing of organic peroxides with peroxidase–modified electrodes," *Analytica Chimica Acta.* 254:81–88 (1991).
Wang, D. L. et al., "Miniaturized Flexible Amperometric Lactate Probe," *Analytical Chemistry,* 65(8):1069–1073 (Apr. 15, 1993).
Wang, J. et al., "Screen–Printable Sol–Gel Enzyme–Containing Carbon Inks," *Analytical Chemistry,* 68(15):2705–2708 (Aug. 1, 1996).
Wang, J. et al., "Sol–Gel–Derived Metal–Dispersed Carbon Composite Amperometric Biosensors," *Electroanalysis,* 9(1):52–55 (1997).
Williams, D.L. et al., "Electrochemical–Enzymatic Analysis of Blood Glucose and Lactate", *Anal. Chem.,* 42(1):118–121 (Jan. 1970).
Wilson, G. S. et al., "Progress toward the Development of an Implantable Sensor for Glucose," *Clinical Chemistry,* 38(9):1613–1617 (1992).
Yabuki, S. et al., "Electro–conductive Enzyme Membrane," *J. Chem. Soc. Commun,* 945–946 (1989).
Yang, I. et al., "Determination of Oxidase Enzyme Substrates Using Cross–Flow Thin–Layer Amperometry," *Electroanalysis,* 8(8–9):716–721 (1996).
Yao, S.J. et al., "The Interference of Ascorbate and Urea in Low–Potential Electrochemical Glucose Sensing", *Proceedings of the Twelfth Annual International Conference of the IEEE Engineering in Medicine and Biology Society,* 12(2):487–489 (Nov. 1–4, 1990).
Yao, T. et al., "A Chemically–Modified Enzyme Membrane Electrode As An Amperometric Glucose Sensor," *Analytica Chimica Acta.,* 148:27–33 (1983).
Ye, L. et al., "High Current Density "Wired" Quinoprotein Glucose Dehydrogenase Electrode," *Anal. Chem.,* 65(3):238–241 (Feb. 1, 1993).
Yildiz, A. et al., "Evaluation of an Improved Thin–Layer Electrode," *Analytical Chemistry,* 40(70):1018–1024 (Jun. 1968).
Zamzow, K. et al., New Wearable Continuous Blood Glucose Monitor (BGM) and Artificial Pancreas (AP), *Diabetes,* 39:5A(20) (May 1990).
Zhang, Y. et al., "Application of cell culture toxicity tests to the development of implantable biosensors," *Biosensors & Bioelectronics,* 6:653–661 (1991).
Zhang, Y. et al., "Elimination of the Acetaminophen Interference in an Implantable Glucose Sensor," *Anal. Chem,* 66:1183–1188 (1994).

* cited by examiner

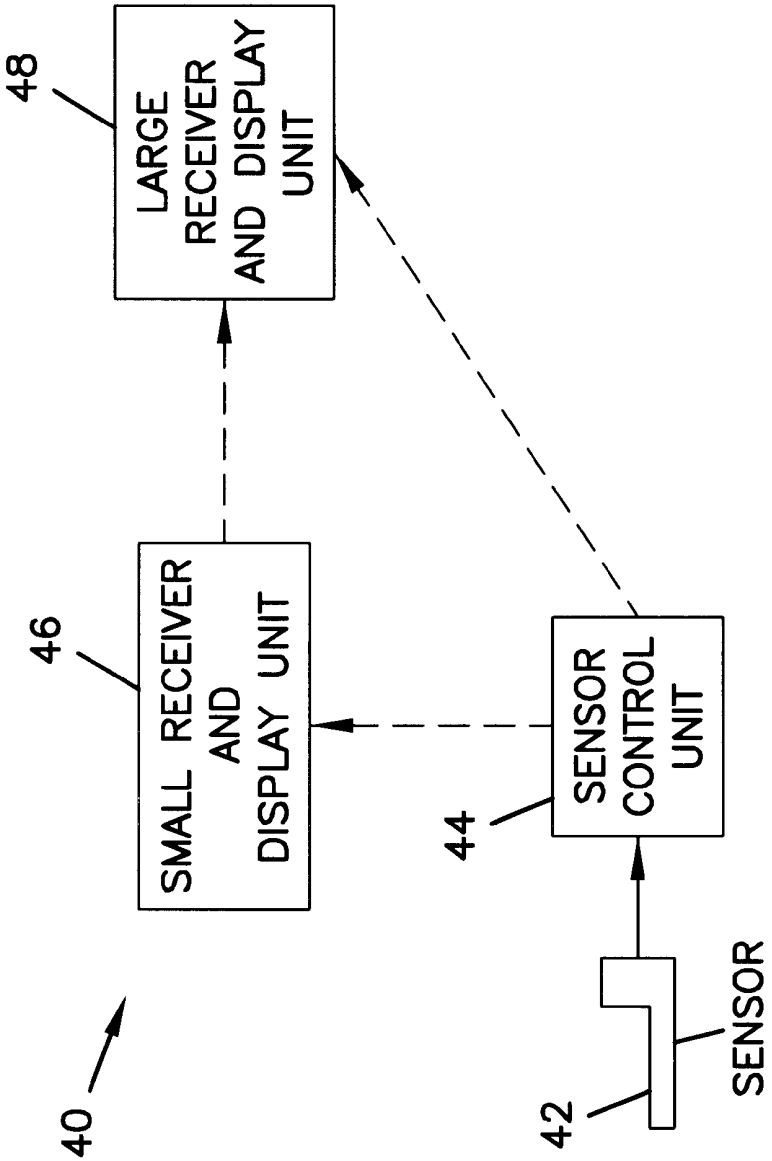

48
LARGE RECEIVER AND DISPLAY UNIT
46
SMALL RECEIVER AND DISPLAY UNIT
44
SENSOR CONTROL UNIT
42
SENSOR
40

FIG. 1

FIG. 2

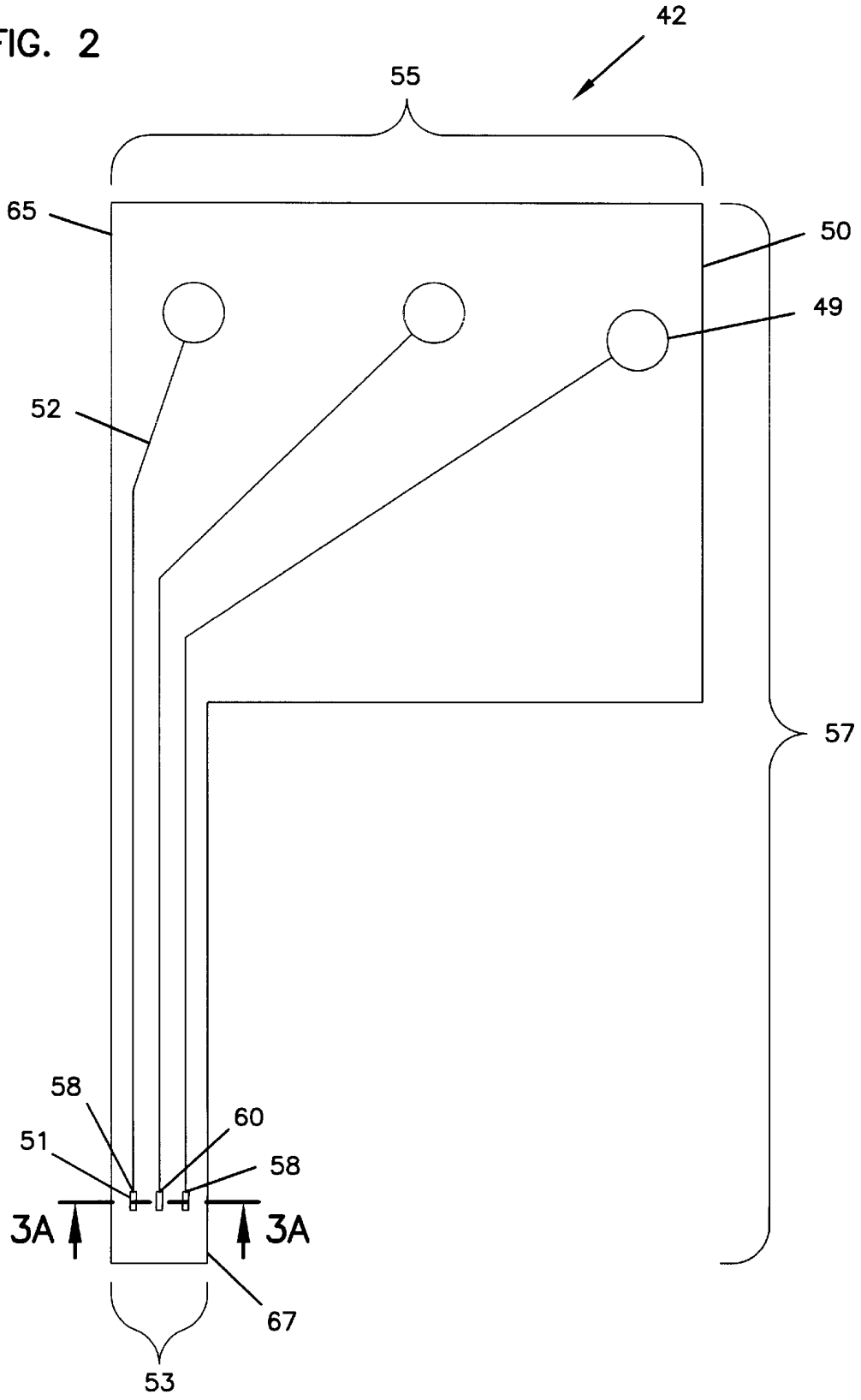


FIG. 3A

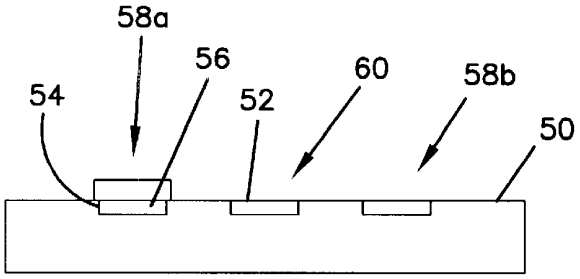


FIG. 3B

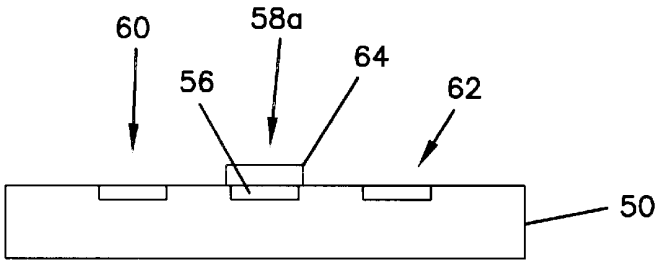


FIG. 9

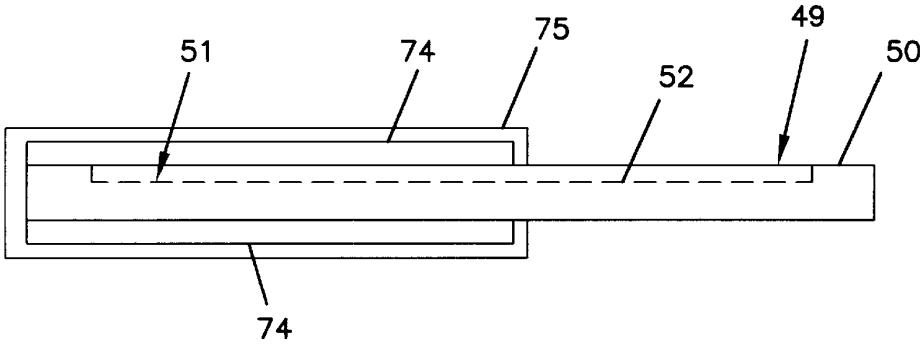


FIG. 4A

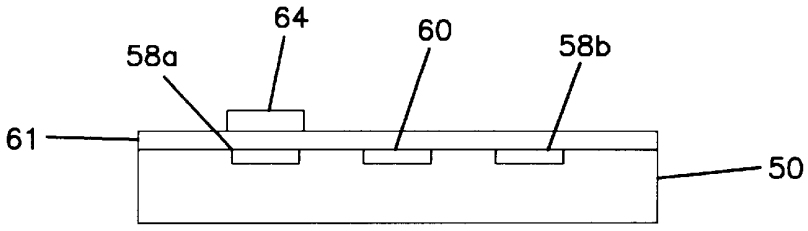


FIG. 4B

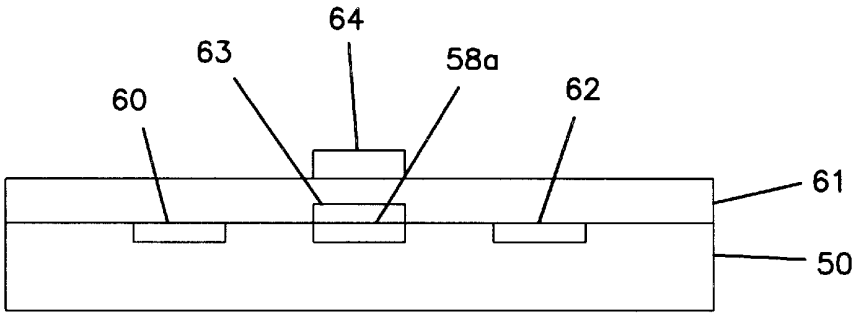


FIG. 5

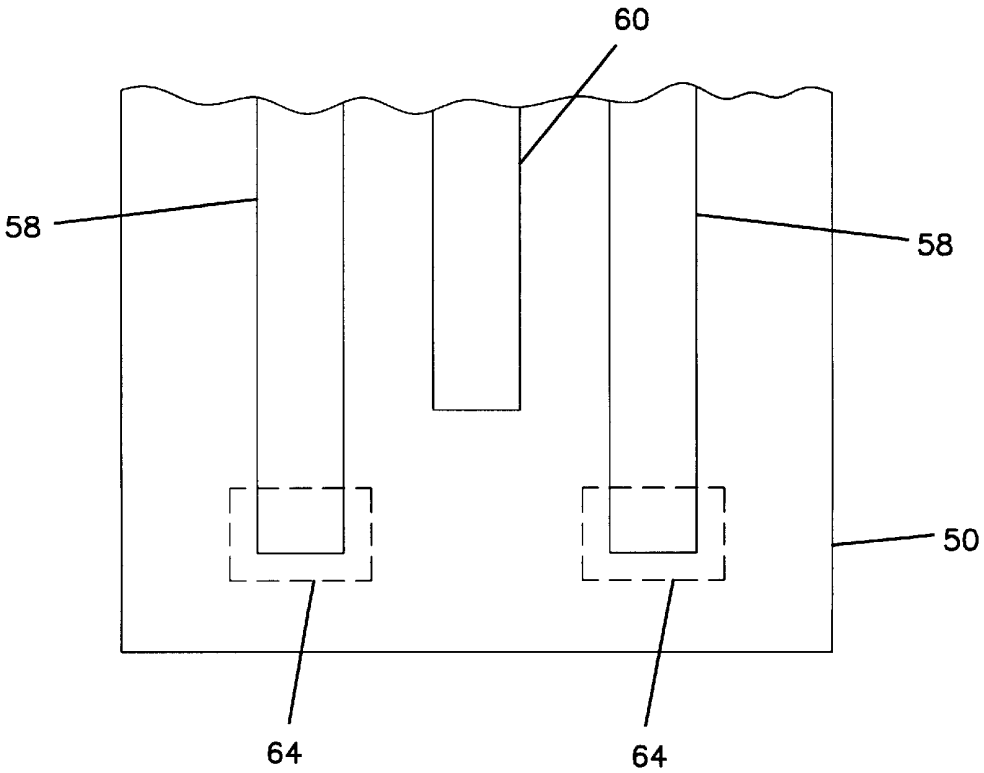


FIG. 6

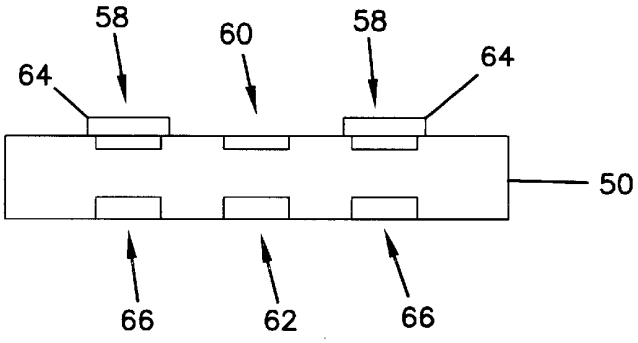


FIG. 7

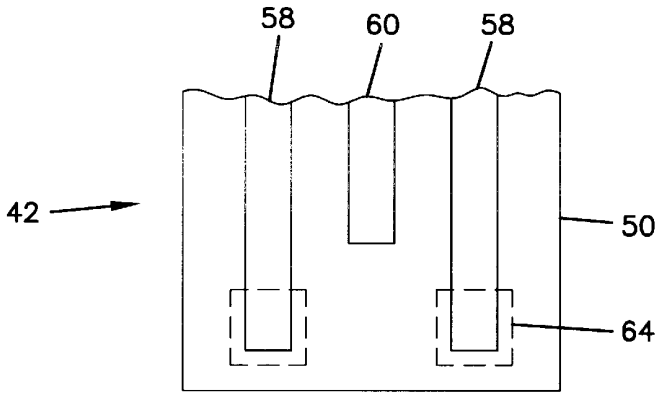


FIG. 8

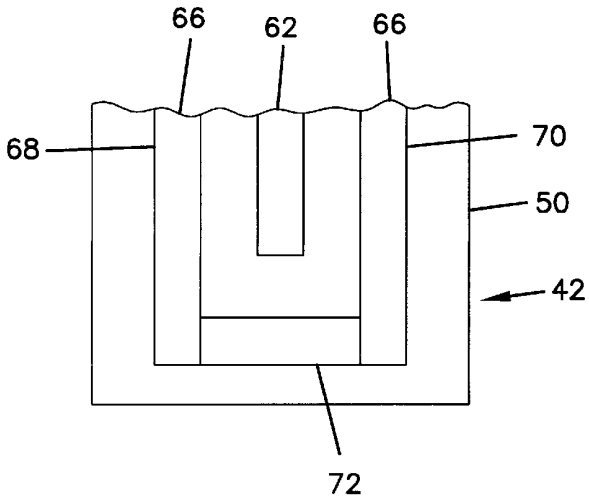


FIG. 10

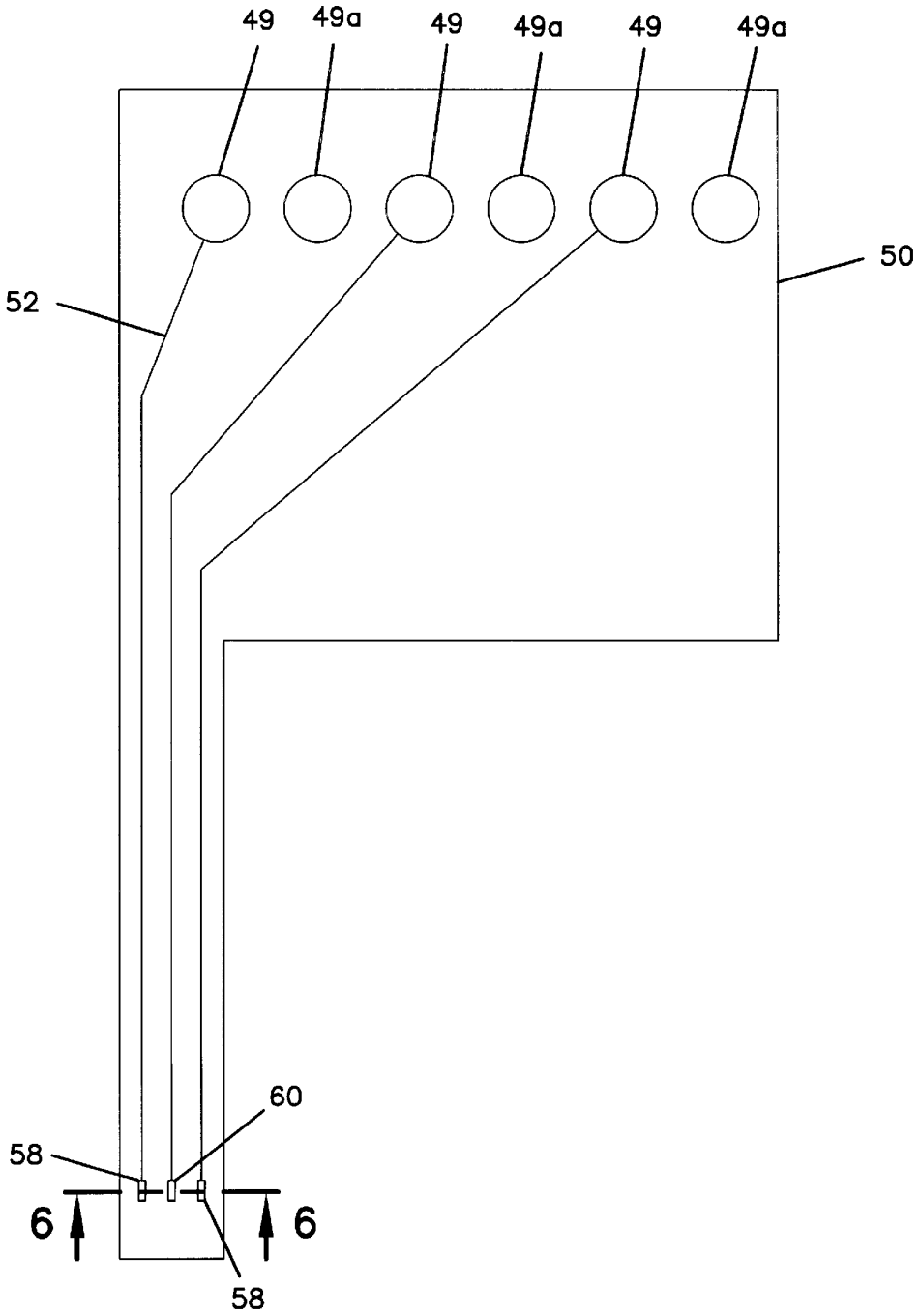


FIG. 11

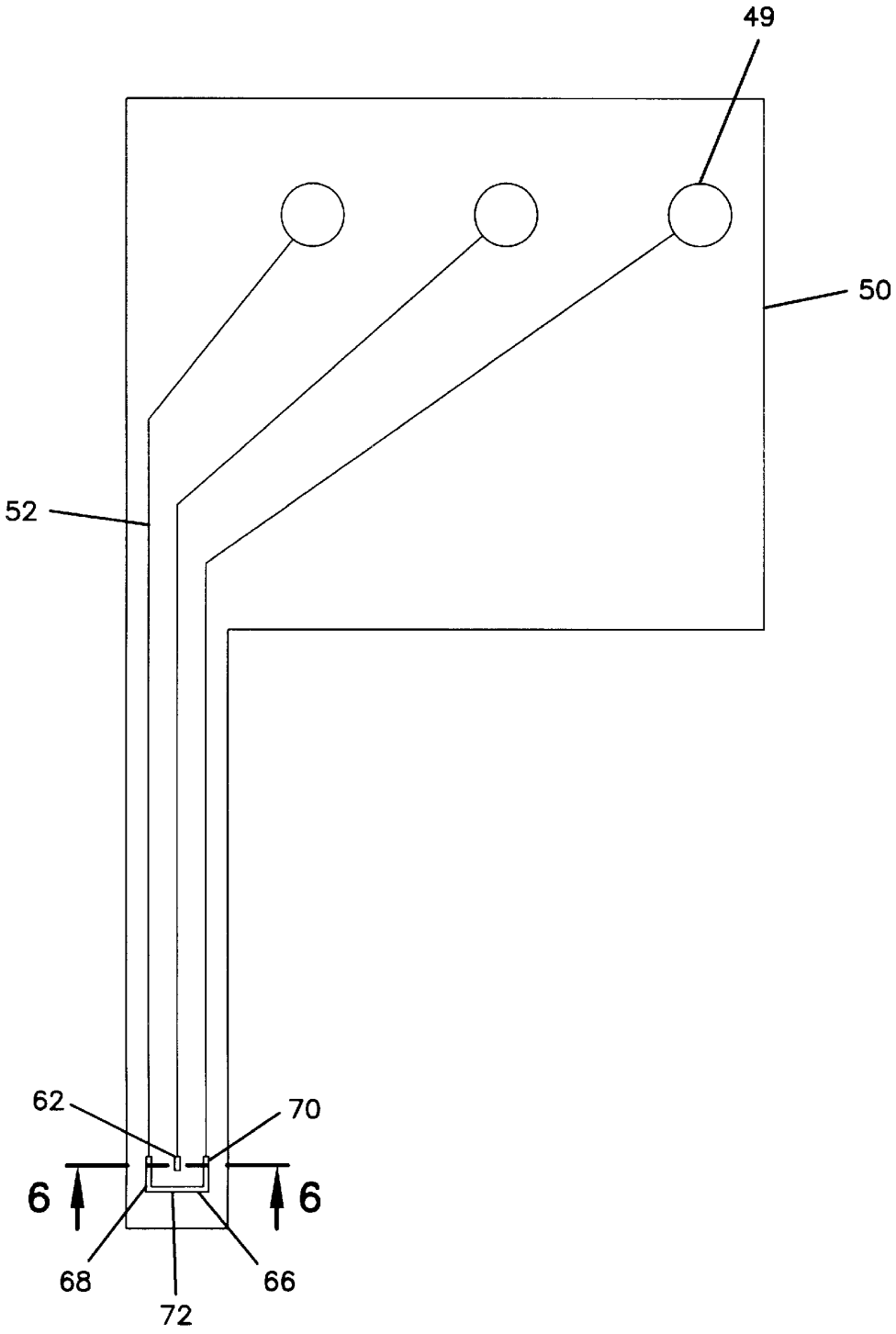


FIG. 12

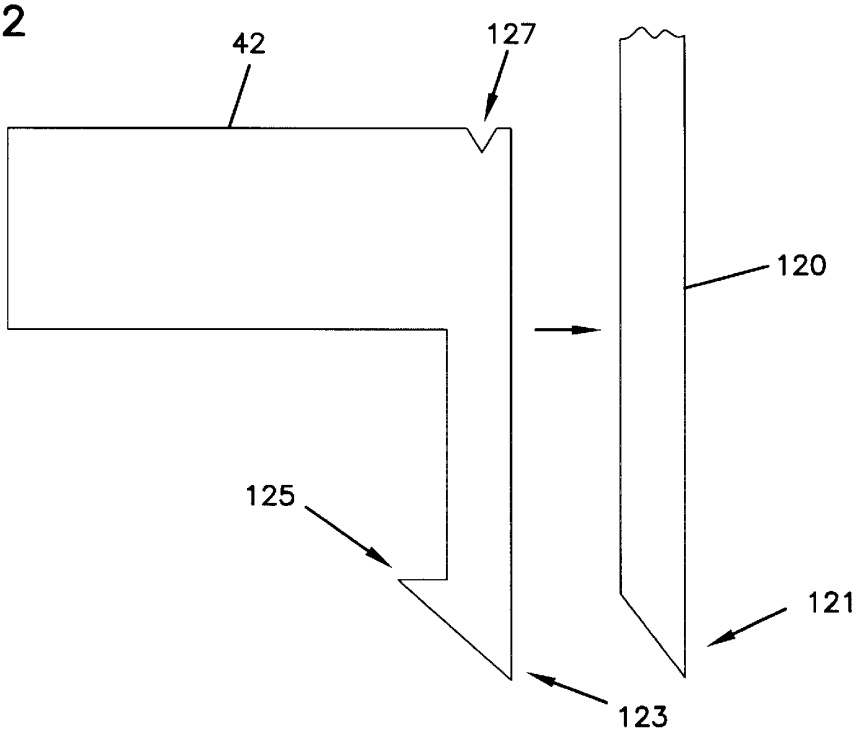


FIG. 13A

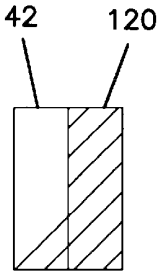


FIG. 13B

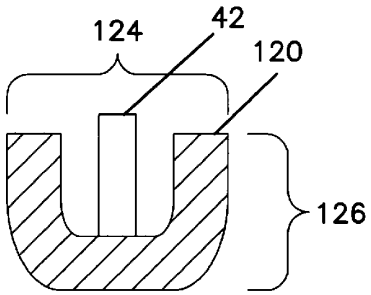


FIG. 13C

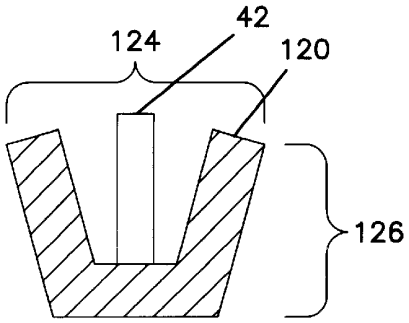


FIG. 15

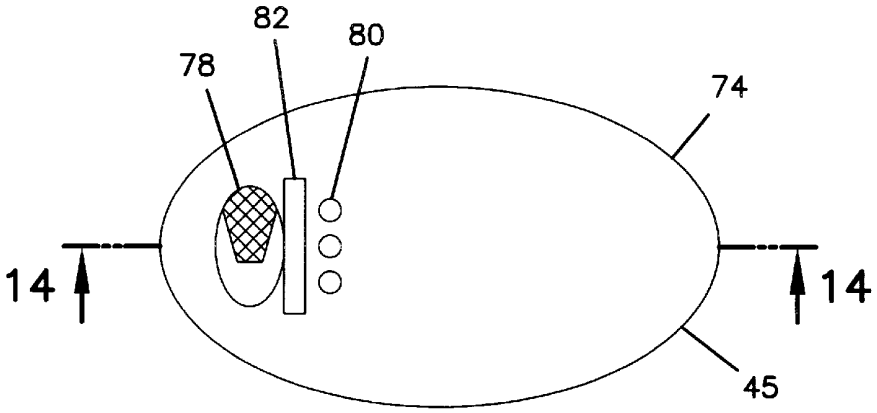


FIG. 16

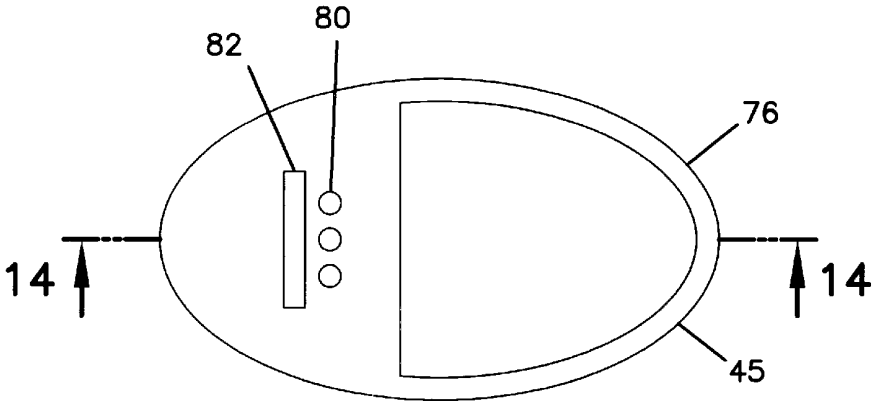


FIG. 14

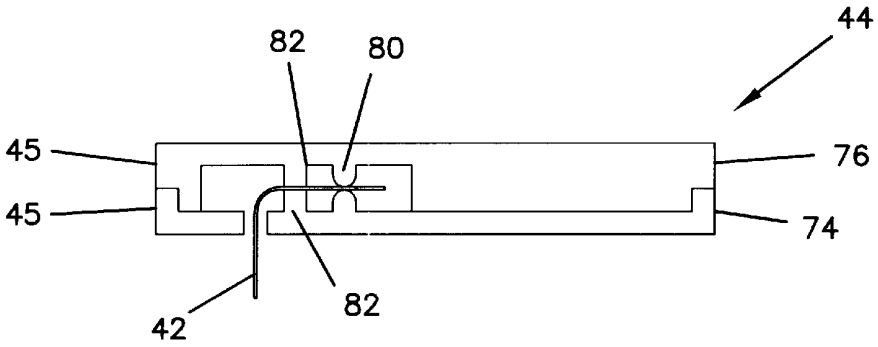
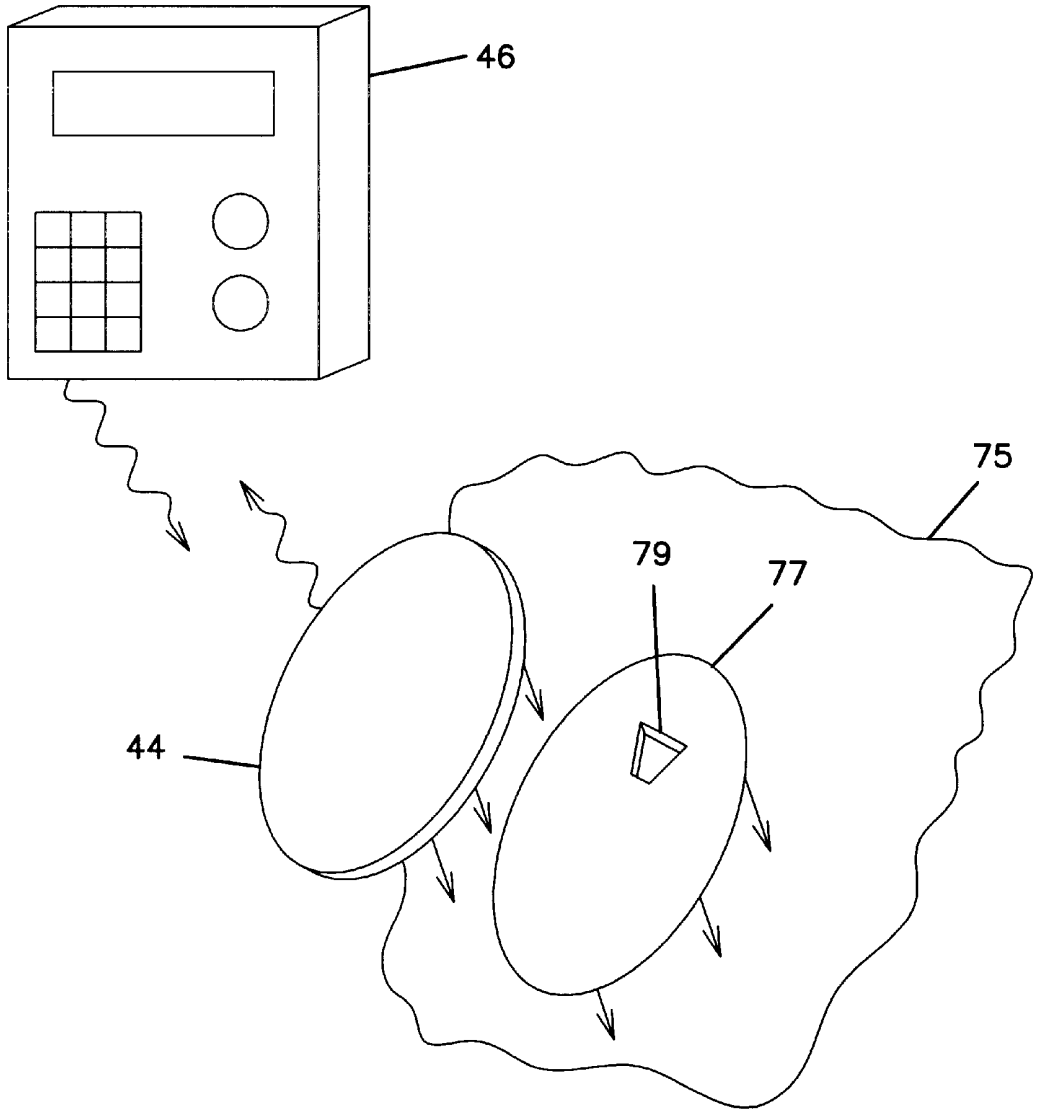


FIG. 17



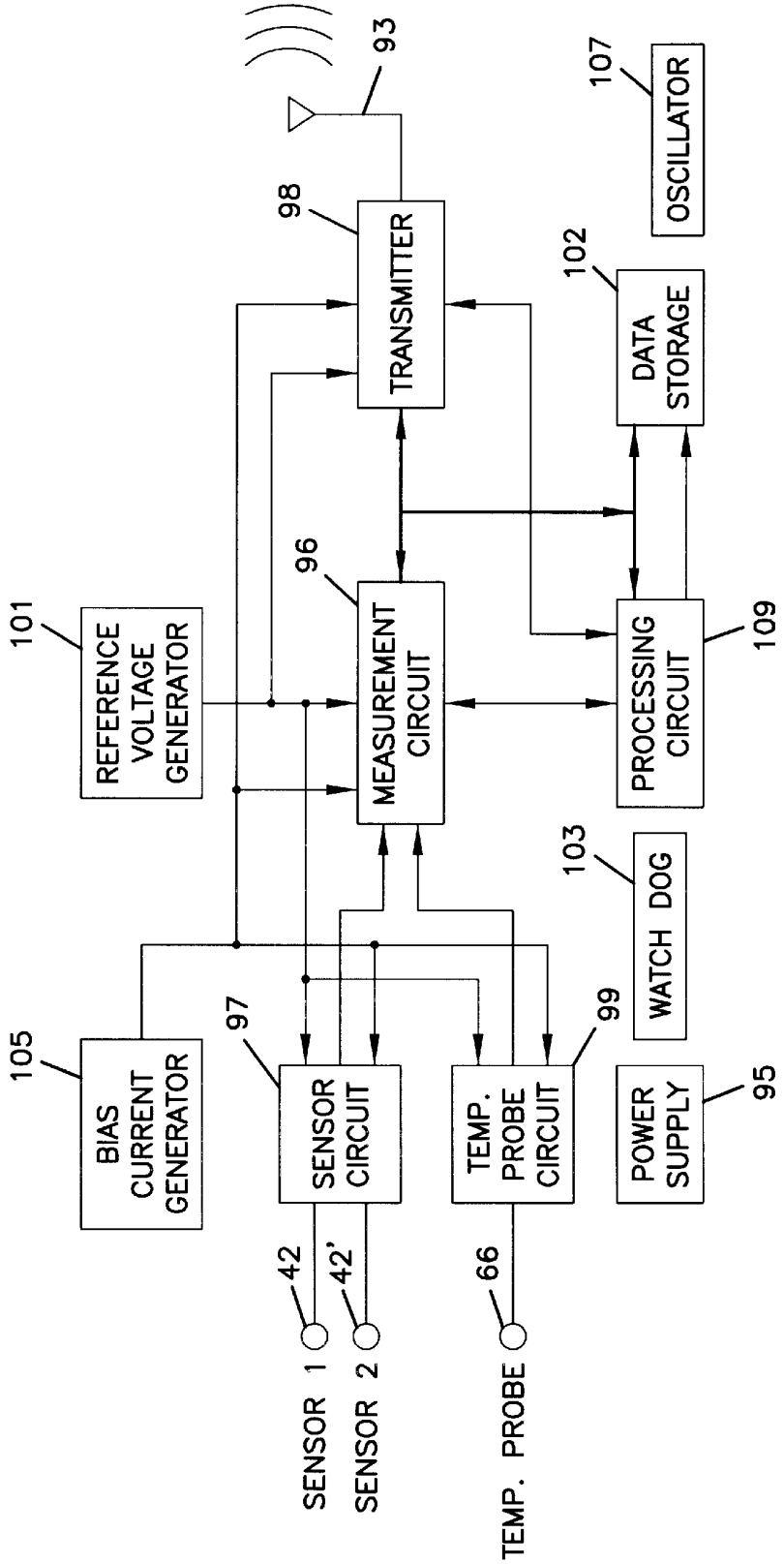

93
98
TRANSMITTER
107
OSCILLATOR
102
DATA STORAGE
96
MEASUREMENT CIRCUIT
101
REFERENCE VOLTAGE GENERATOR
PROCESSING CIRCUIT
109
103
WATCH DOG
105
BIAS CURRENT GENERATOR
97
SENSOR CIRCUIT
99
TEMP. PROBE CIRCUIT
POWER SUPPLY
95
42
42'
66
SENSOR 1
SENSOR 2
TEMP. PROBE

FIG. 18A

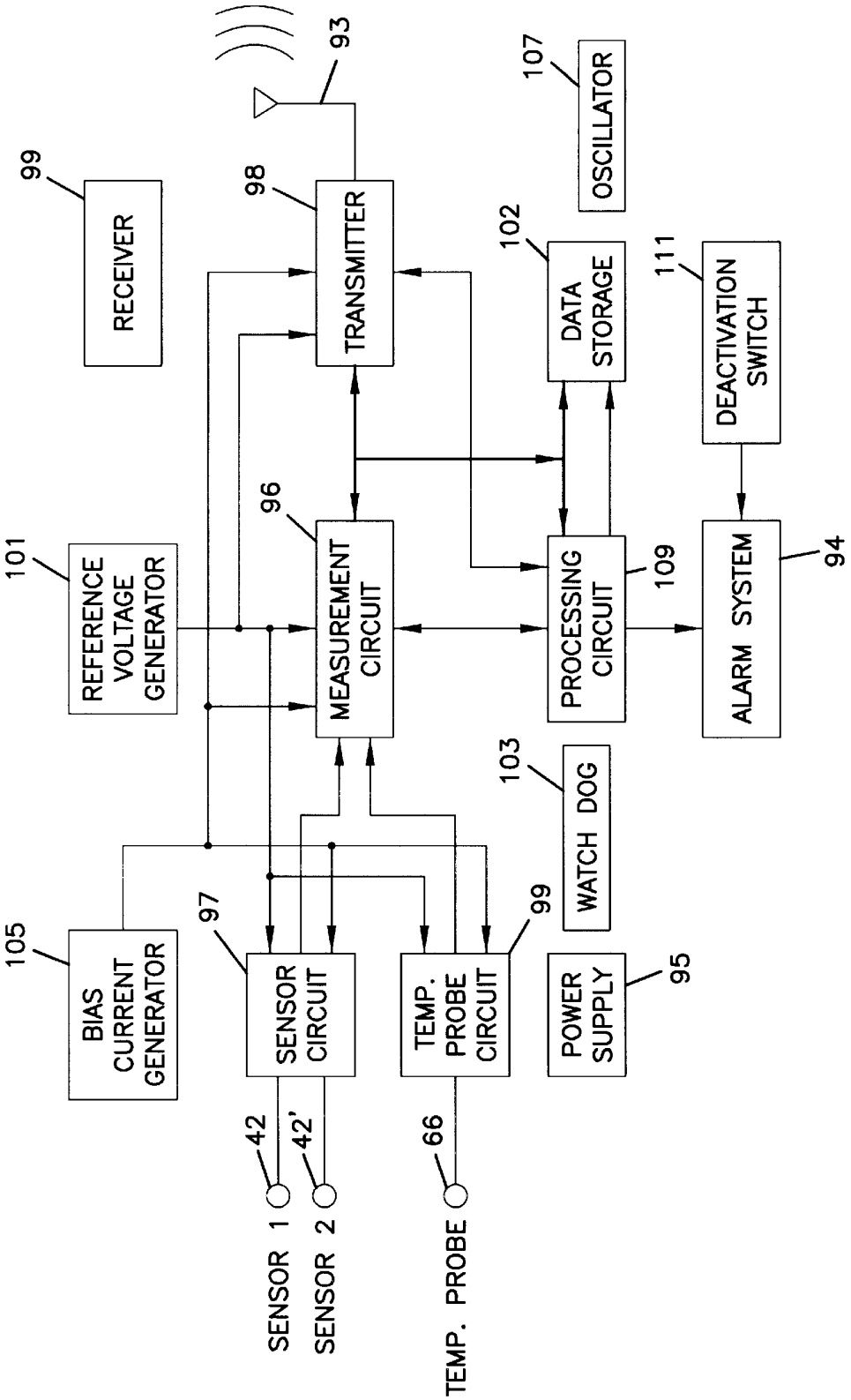

93
107
OSCILLATOR
99
98
102
111
RECEIVER
TRANSMITTER
DATA STORAGE
DEACTIVATION SWITCH
96
101
REFERENCE VOLTAGE GENERATOR
MEASUREMENT CIRCUIT
PROCESSING CIRCUIT
109
ALARM SYSTEM
94
103
WATCH DOG
105
97
99
BIAS CURRENT GENERATOR
SENSOR CIRCUIT
TEMP. PROBE CIRCUIT
POWER SUPPLY
95
42
42'
66
SENSOR 1
SENSOR 2
TEMP. PROBE

FIG. 18B

FIG. 19A

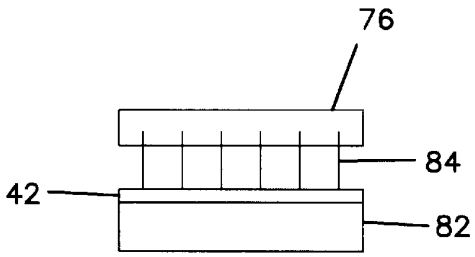


FIG. 19B

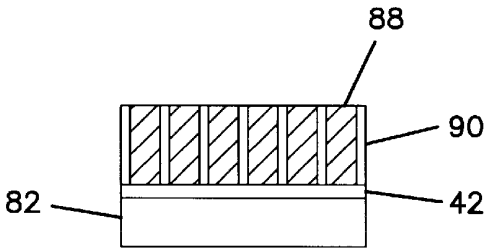


FIG. 19C

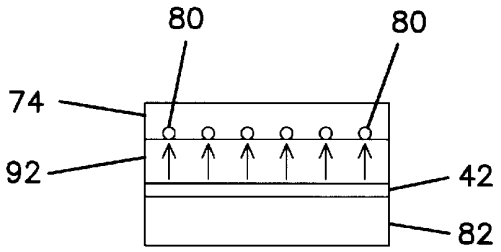


FIG. 19D

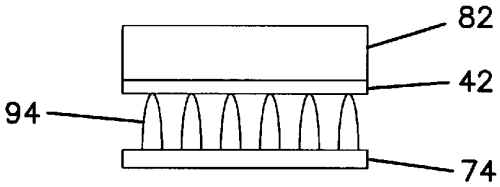


FIG. 19E

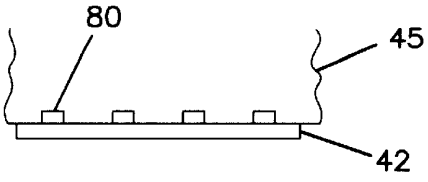


FIG. 19F

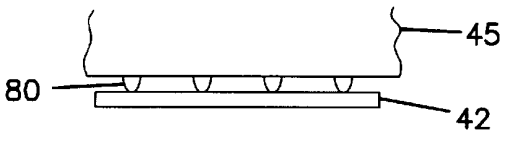


FIG. 20A

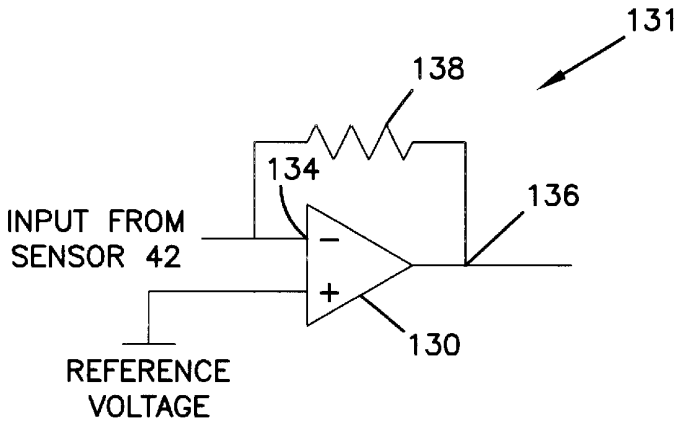
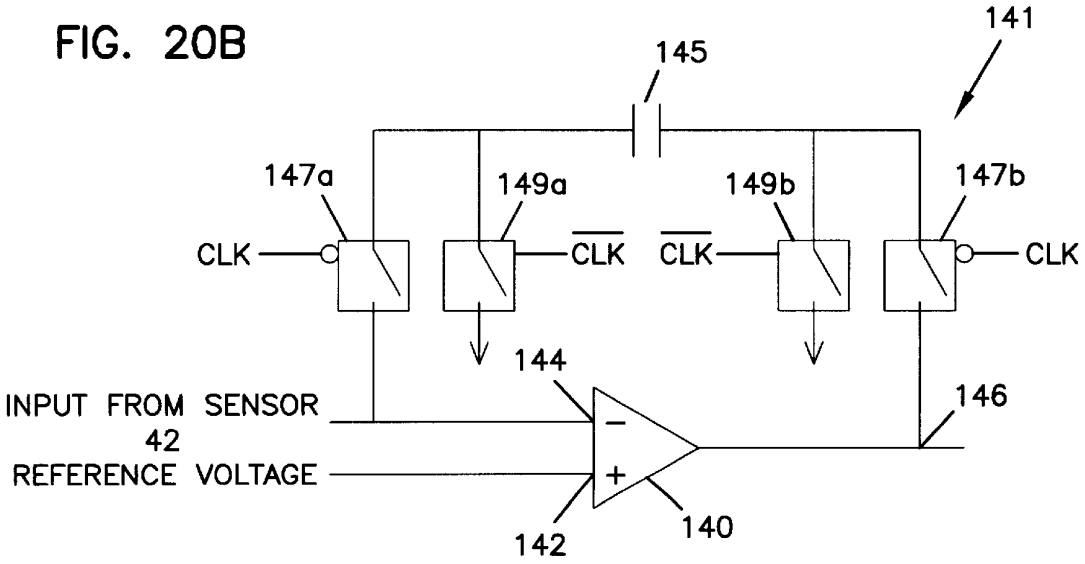


FIG. 20B



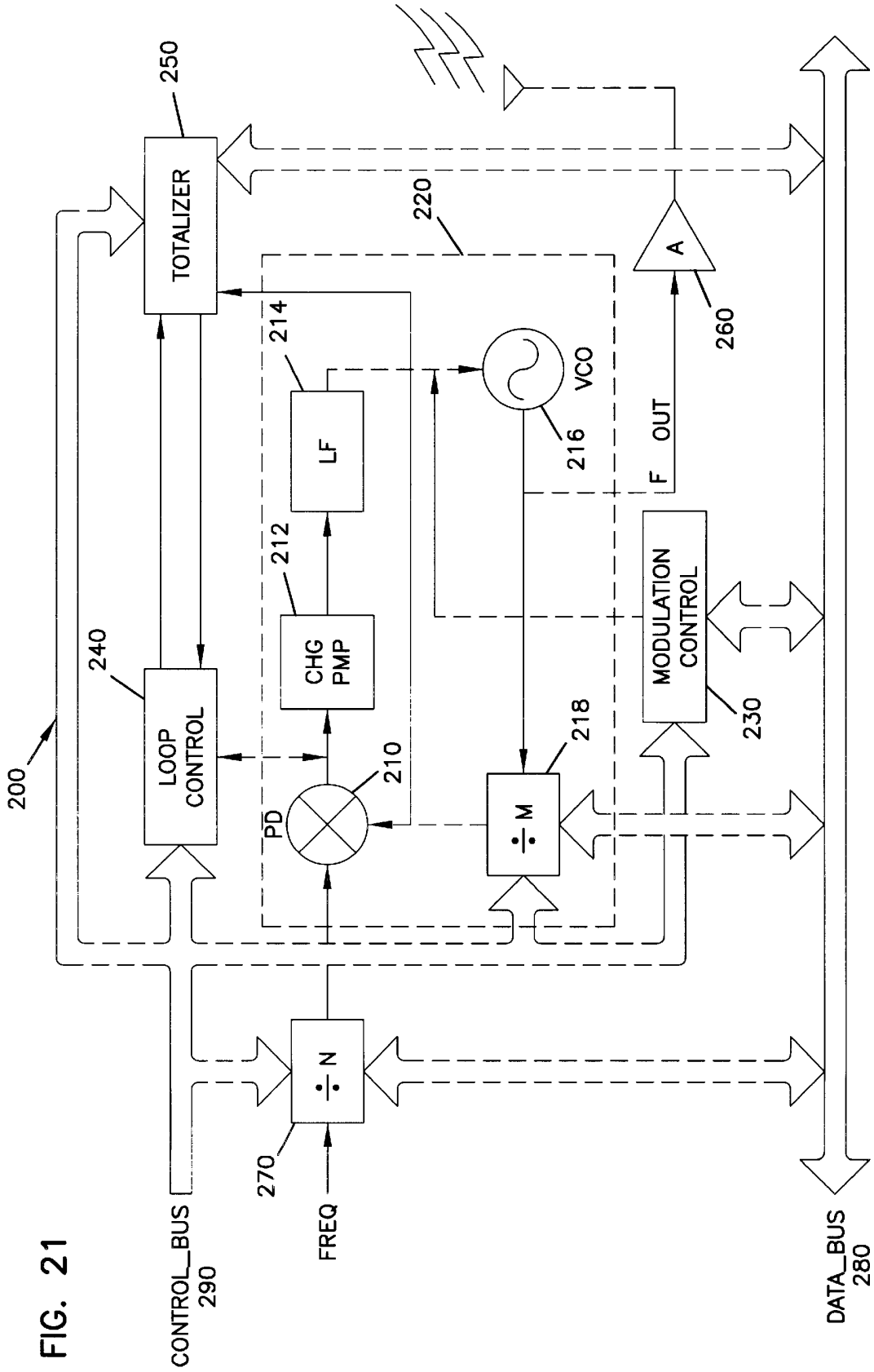

250
TOTALIZER
220
A
260
214
LF
VCO
216
F OUT
212
CHG PMP
MODULATION CONTROL
240
LOOP CONTROL
230
200
218
210
PD
÷ M
÷ N
270
FREQ
CONTROL_BUS 290
DATA_BUS 280

FIG. 21

FIG. 22

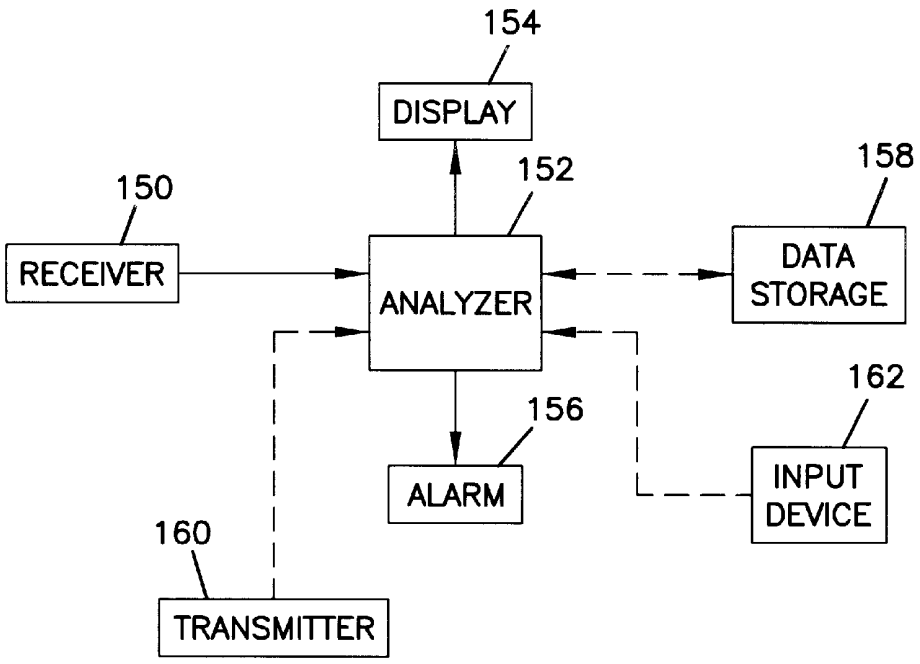

154
DISPLAY
152
158
150
RECEIVER
ANALYZER
DATA STORAGE
162
156
ALARM
INPUT DEVICE
160
TRANSMITTER

FIG. 23

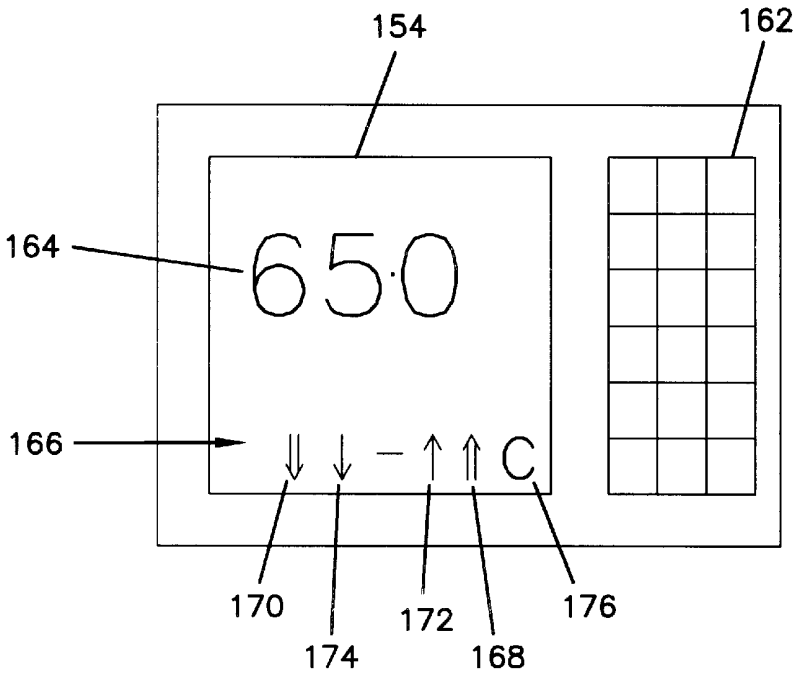
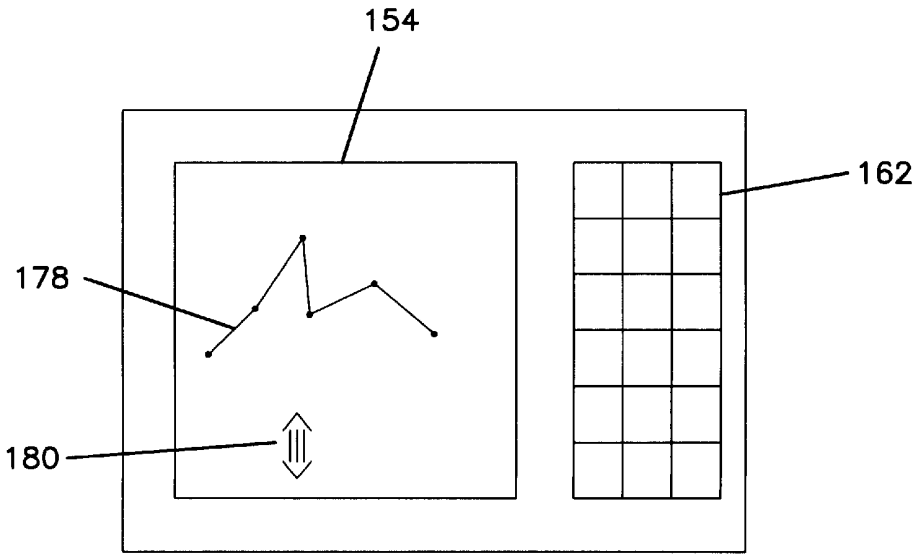


FIG. 24



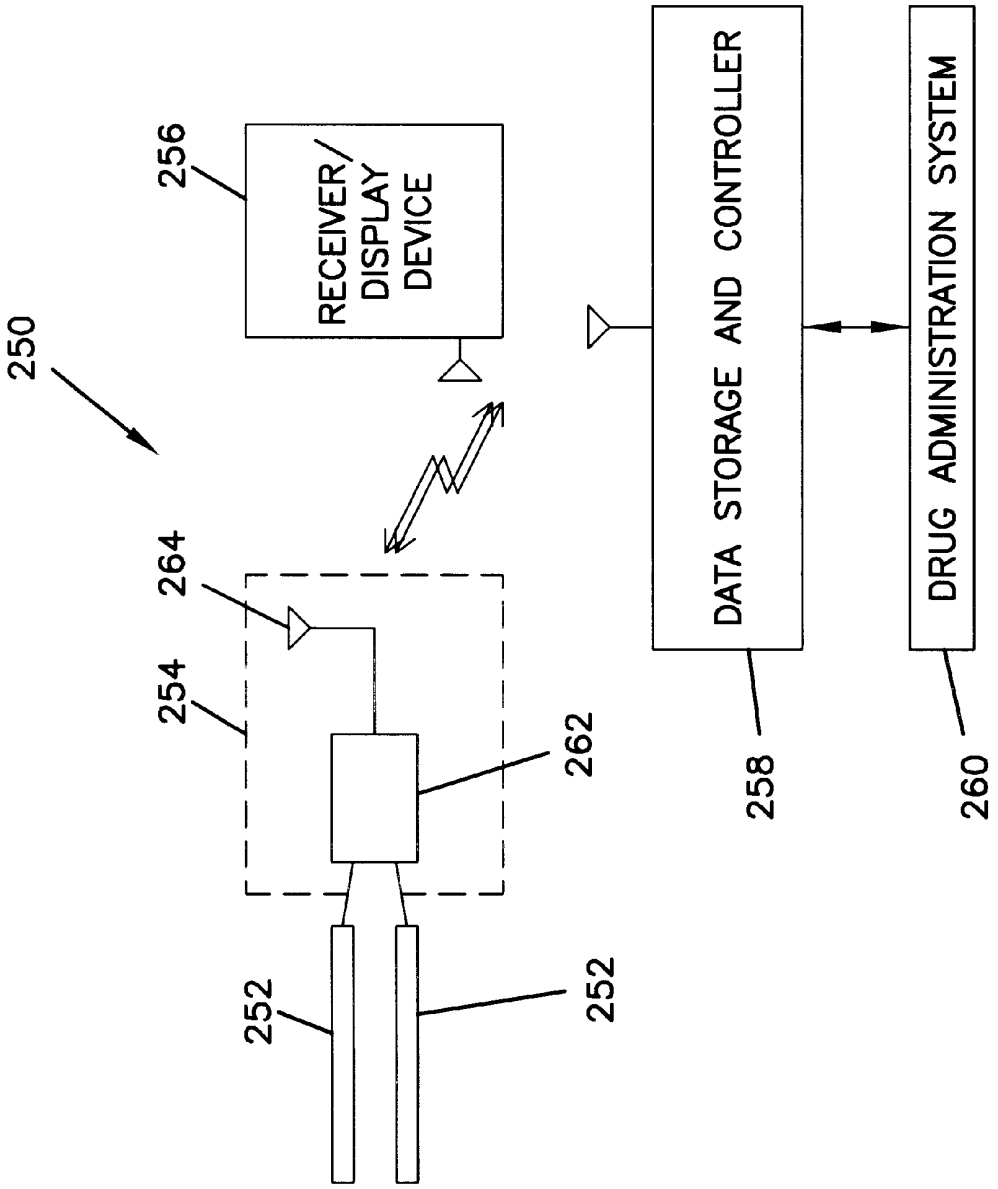

250
256
RECEIVER/ DISPLAY DEVICE
264
254
262
252
252
DATA STORAGE AND CONTROLLER
DRUG ADMINISTRATION SYSTEM
258
260

FIG. 25

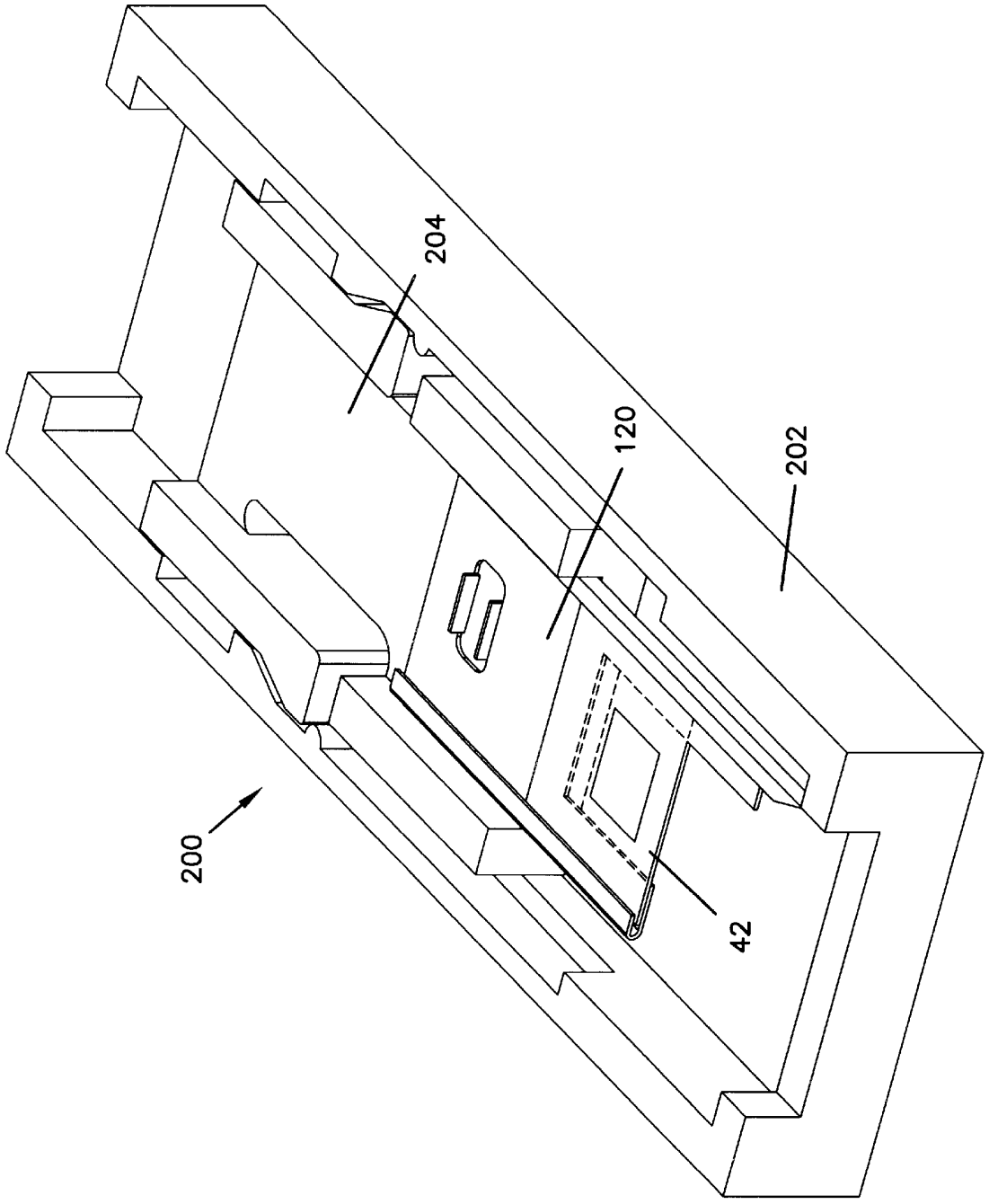


FIG. 26

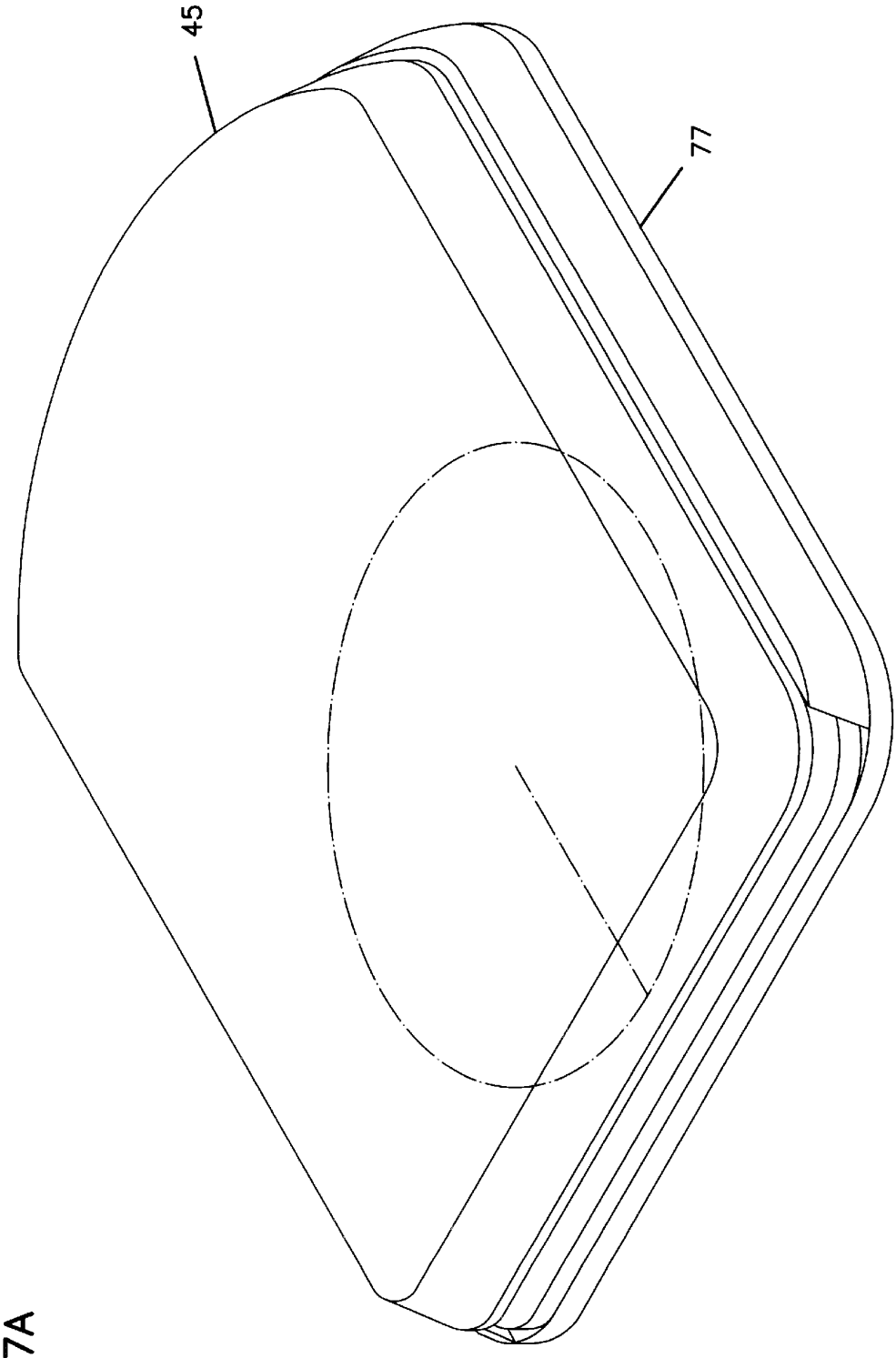


FIG. 27A

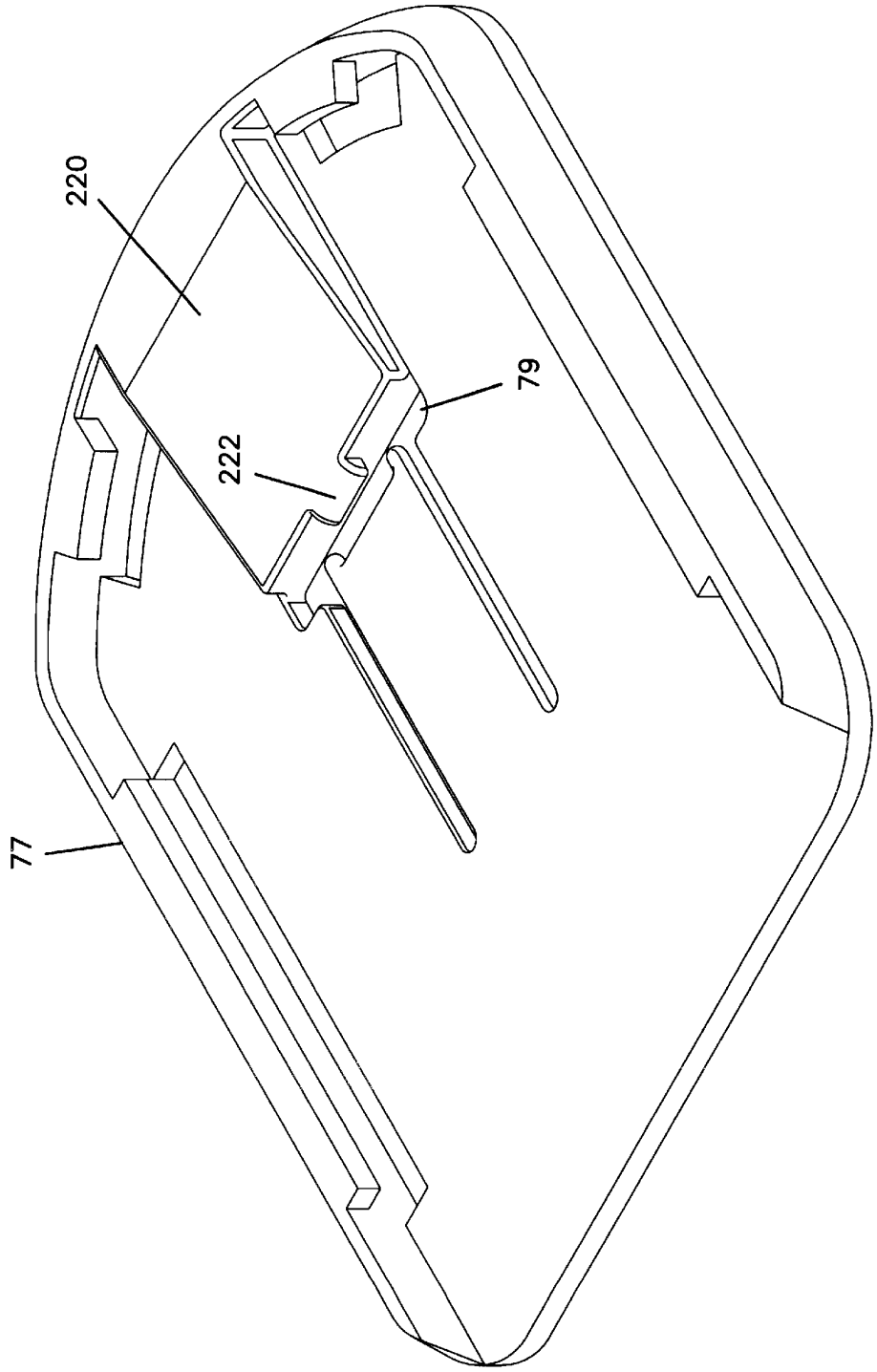


FIG. 27B

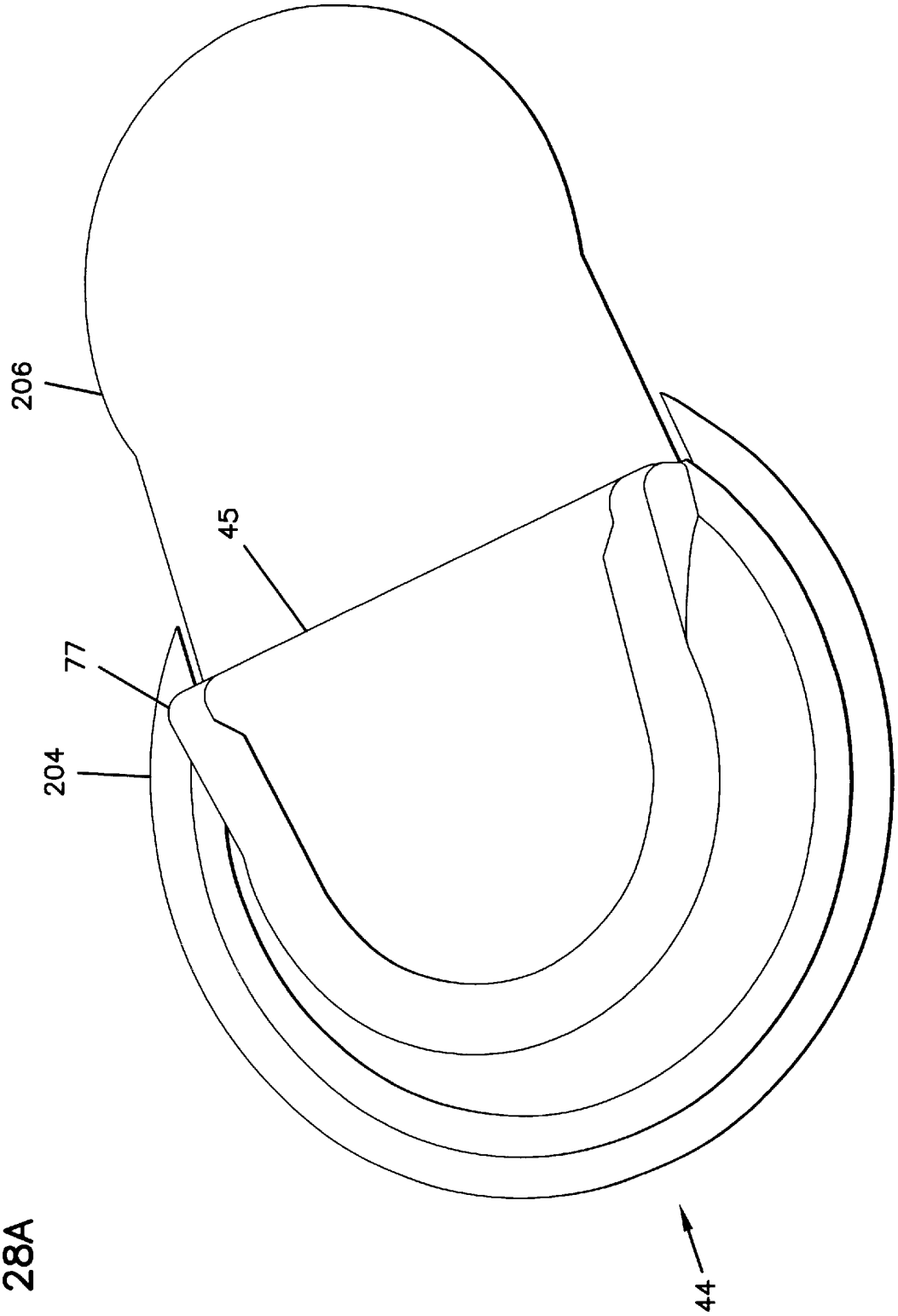


FIG. 28A

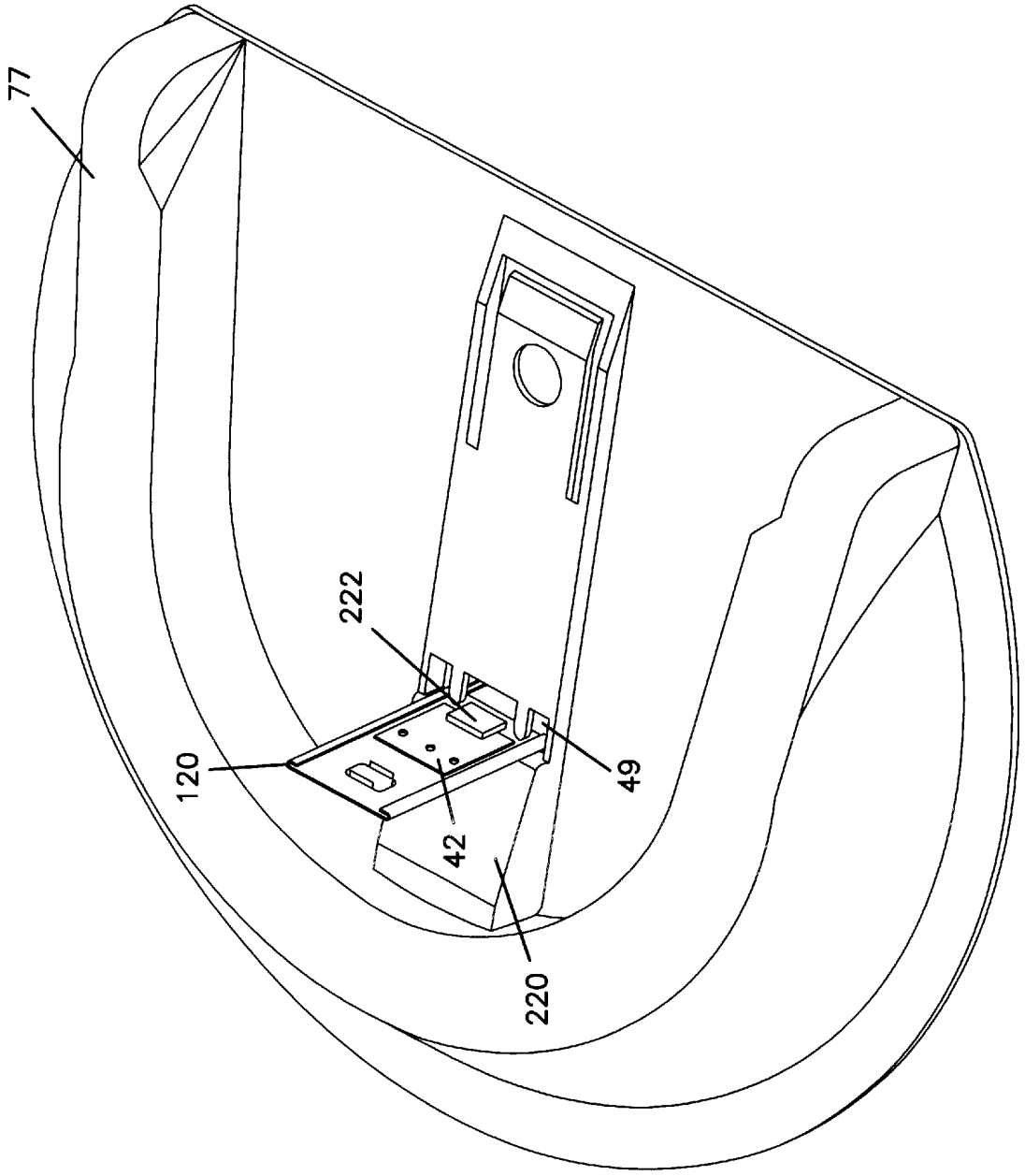


FIG. 28B

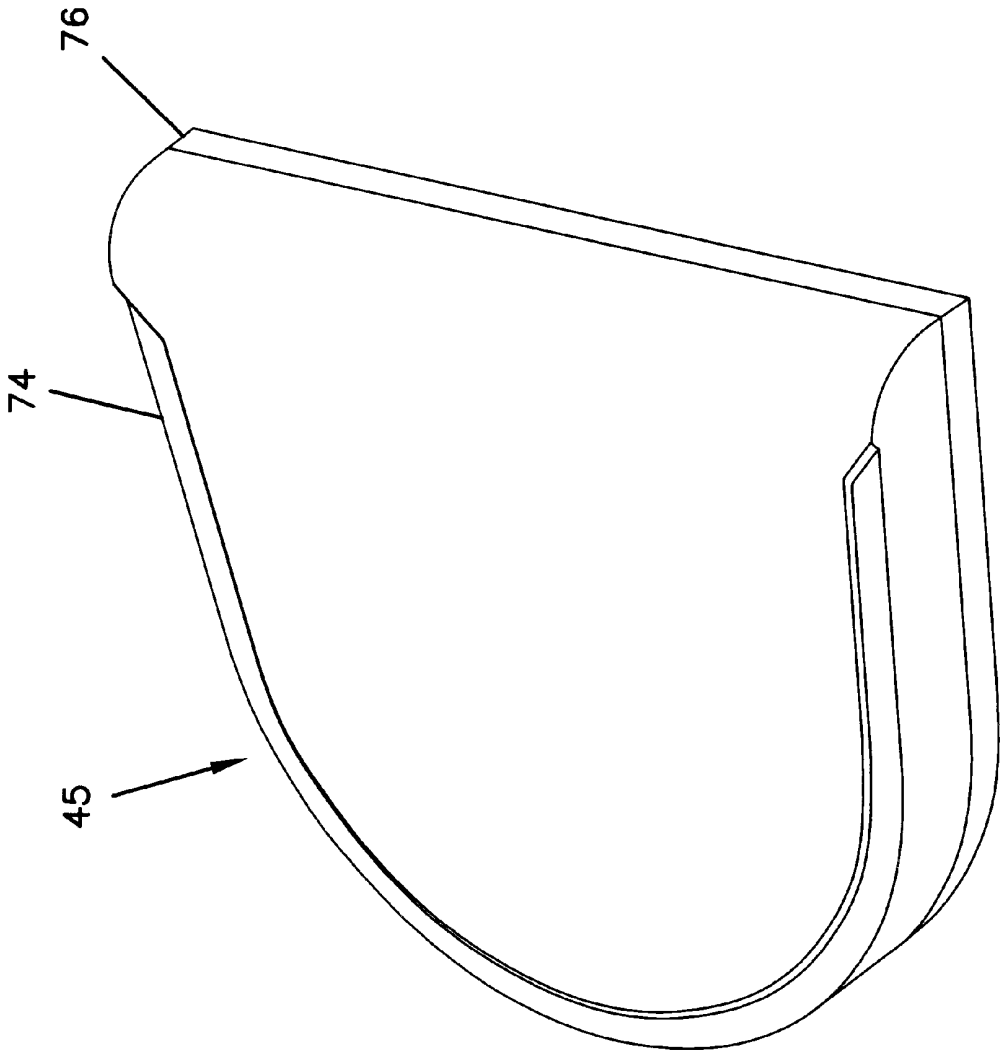


FIG. 28C

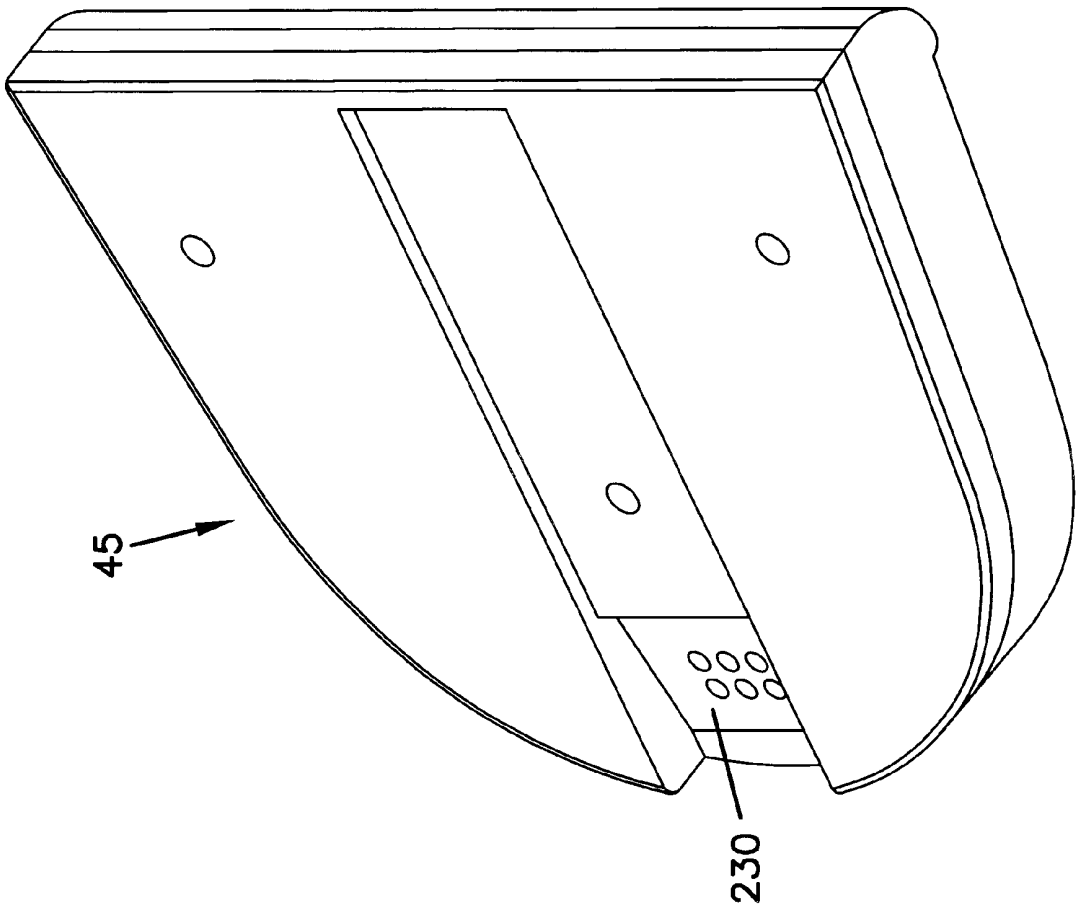


FIG. 28D

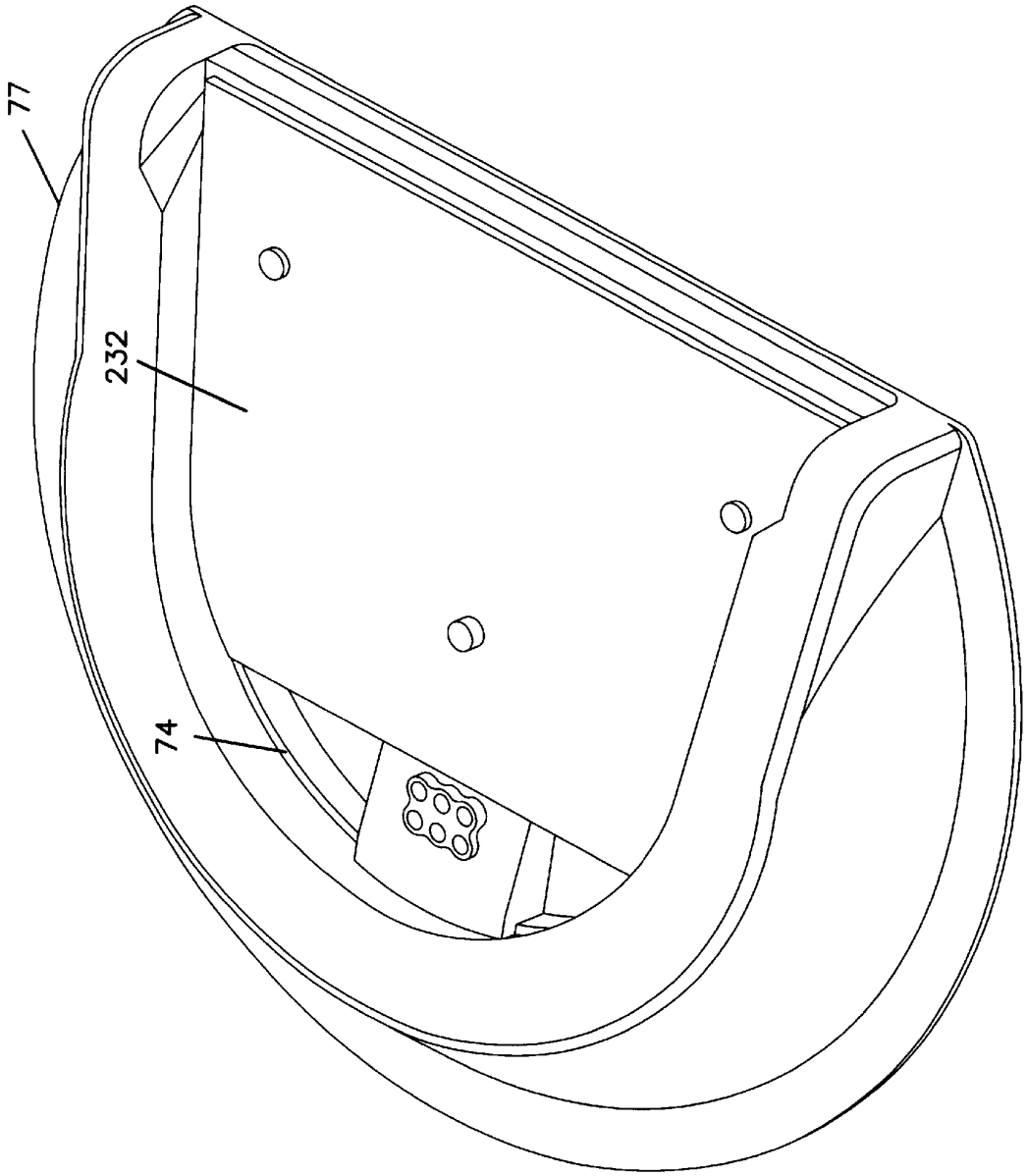


FIG. 28E

ANALYTE MONITORING DEVICE AND METHODS OF USE

FIELD OF THE INVENTION

The present invention is, in general, directed to devices and methods for the in vivo monitoring of an analyte, such as glucose or lactate. More particularly, the present invention relates to devices and methods for the in vivo monitoring of an analyte using an electrochemical sensor to provide information to a patient about the level of the analyte.

BACKGROUND OF THE INVENTION

The monitoring of the level of glucose or other analytes, such as lactate or oxygen, in certain individuals is vitally important to their health. High or low levels of glucose or other analytes may have detrimental effects. The monitoring of glucose is particularly important to individuals with diabetes, as they must determine when insulin is needed to reduce glucose levels in their bodies or when additional glucose is needed to raise the level of glucose in their bodies.

A conventional technique used by many diabetics for personally monitoring their blood glucose level includes the periodic drawing of blood, the application of that blood to a test strip, and the determination of the blood glucose level using calorimetric, electrochemical, or photometric detection. This technique does not permit continuous or automatic monitoring of glucose levels in the body, but typically must be performed manually on a periodic basis. Unfortunately, the consistency with which the level of glucose is checked varies widely among individuals. Many diabetics find the periodic testing inconvenient and they sometimes forget to test their glucose level or do not have time for a proper test. In addition, some individuals wish to avoid the pain associated with the test. These situations may result in hyperglycemic or hypoglycemic episodes. An in vivo glucose sensor that continuously or automatically monitors the individual's glucose level would enable individuals to more easily monitor their glucose, or other analyte, levels.

A variety of devices have been developed for continuous or automatic monitoring of analytes, such as glucose, in the blood stream or interstitial fluid. A number of these devices use electrochemical sensors which are directly implanted into a blood vessel or in the subcutaneous tissue of a patient. However, these devices are often difficult to reproducibly and inexpensively manufacture in large numbers. In addition, these devices are typically large, bulky, and/or inflexible, and many can not be used effectively outside of a controlled medical facility, such as a hospital or a doctor's office, unless the patient is restricted in his activities.

Some devices include a sensor guide which rests on or near the skin of the patient and may be attached to the patient to hold the sensor in place. These sensor guides are typically bulky and do not allow for freedom of movement. In addition, the sensor guides or the sensors include cables or wires for connecting the sensor to other equipment to direct the signals from the sensors to an analyzer. The size of the sensor guides and presence of cables and wires hinders the convenient use of these devices for everyday applications. There is a need for a small, compact device that can operate the sensor and provide signals to an analyzer without substantially restricting the movements and activities of a patient.

The patient's comfort and the range of activities that can be performed while the sensor is implanted are important considerations in designing extended-use sensors for continuous or automatic in vivo monitoring of the level of an analyte, such as glucose. There is a need for a small, comfortable device which can continuously monitor the level of an analyte, such as glucose, while still permitting the patient to engage in normal activities. Continuous and/or automatic monitoring of the analyte can provide a warning to the patient when the level of the analyte is at or near a threshold level. For example, if glucose is the analyte, then the monitoring device might be configured to warn the patient of current or impending hyperglycemia or hypoglycemia. The patient can then take appropriate actions.

SUMMARY OF THE INVENTION

Generally, the present invention relates to methods and devices for the continuous and/or automatic in vivo monitoring of the level of an analyte using a subcutaneously implantable sensor. Many of these devices are small and comfortable when used, thereby allowing a wide range of activities. One embodiment is a sensor control unit having a housing adapted for placement on skin. The housing is also adapted to receive a portion of an electrochemical sensor. The sensor control unit includes two or more conductive contacts disposed on the housing and configured for coupling to two or more contact pads on the sensor. A transmitter is disposed in the housing and coupled to the plurality of conductive contacts for transmitting data obtained using the sensor. The sensor control unit may also include a variety of optional components, such as, for example, adhesive for adhering to the skin, a mounting unit, a receiver, a processing circuit, a power supply (e.g., a battery), an alarm system, a data storage unit, a watchdog circuit, and a measurement circuit. Other optional components are described below.

Another embodiment of the invention is a sensor assembly that includes the sensor control unit described above. The sensor assembly also includes a sensor having at least one working electrode and at least one contact pad coupled to the working electrode or electrodes. The sensor may also include optional components, such as, for example, a counter electrode, a counter/reference electrode, a reference electrode, and a temperature probe. Other components and options for the sensor are described below.

A further embodiment of the invention is an analyte monitoring system that includes the sensor control unit described above. The analyte monitoring system also includes a sensor that has at least one working electrode and at least one contact pad coupled to the working electrode or electrodes. The analyte monitoring system also includes a display unit that has a receiver for receiving data from the sensor control unit and a display coupled to the receiver for displaying an indication of the level of an analyte. The display unit may optionally include a variety of components, such as, for example, a transmitter, an analyzer, a data storage unit, a watchdog circuit, an input device, a power supply, a clock, a lamp, a pager, a telephone interface, a computer interface, an alarm or alarm system, a radio, and a calibration unit. Further components and options for the display unit are described below. In addition, the analyte monitoring system or a component of the analyte monitoring system may optionally include a processor capable of determining a drug or treatment protocol and/or a drug delivery system.

Yet another embodiment of the invention is an insertion kit for inserting an electrochemical sensor into a patient. The insertion kit includes an inserter. A portion of the inserter has a sharp, rigid, planer structure adapted to support the sensor during insertion of the electrochemical sensor. The insertion kit also includes an insertion gun having a port configured to

accept the electrochemical sensor and the inserter. The insertion gun has a driving mechanism for driving the inserter and electrochemical sensor into the patient, and a retraction mechanism for removing the inserter while leaving the sensor within the patient.

Another embodiment is a method of using an electrochemical sensor. A mounting unit is adhered to skin of a patient. An insertion gun is aligned with a port on the mounting unit. The electrochemical sensor is disposed within the insertion gun and then the electrochemical sensor is inserted into the skin of the patient using the insertion gun. The insertion gun is removed and a housing of the sensor control unit is mounted on the mounting base. A plurality of conductive contacts disposed on the housing is coupled to a plurality of contact pads disposed on the electrochemical sensor to prepare the sensor for use.

One embodiment of the invention is a method for detecting failures in an implanted analyte-responsive sensor. An analyte-responsive sensor is implanted into a patient. The analyte-responsive sensor includes N working electrodes, where N is an integer and is two or greater, and a common counter electrode. Signals generated at one of the N working electrodes and at the common counter electrode are then obtained and the sensor is determined to have failed if the signal from the common counter electrode is not N times the signal from one of the working electrodes, within a predetermined threshold limit.

Yet another embodiment is a method of calibrating an electrochemical sensor having one or more working electrodes implanted in a patient. A signal is generated from each of the working electrodes. Several conditions are tested to determine if calibration is appropriate. First, the signals from each of the one or more working electrodes should differ by less than a first threshold amount. Second, the signals from each of the one or more working electrodes should be within a predetermined range. And, third, a rate of change of the signals from each of the one or more working electrodes should be less than a second threshold amount. A calibration value is found assaying a calibration sample of a patient's body fluid. The calibration value is then related to at least one of the signals from the one or more working electrodes if the conditions described above are met.

A further embodiment is a method for monitoring a level of an analyte. A sensor is inserted into a skin of a patient and a sensor control unit is attached to the skin of the patient. Two or more conductive contacts on the sensor control unit are coupled to contact pads on the sensor. Then, using the sensor control unit, data is collected regarding a level of an analyte from signals generated by the sensor. The collected data is transmitted to a display unit and an indication of the level of the analyte is displayed on the display unit.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The Figures and the detailed description which follow more particularly exemplify these embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be more completely understood in consideration of the following detailed description of various embodiments of the invention in connection with the accompanying drawings, in which:

FIGS. **1**, **1**A, **1**B is a block diagram of one embodiment of a subcutaneous analyte monitor using a subcutaneously implantable analyte sensor, according to the invention;

FIG. **2** is a top view of one embodiment of an analyte sensor, according to the invention;

FIGS. **3**, **3**A is a cross-sectional view of the analyte sensor of FIG. **2**;

FIG. **3**B is a cross-sectional view of another embodiment of an analyte sensor, according to the invention;

FIG. **4**A is a cross-sectional view of a third embodiment of an analyte sensor, according to the invention;

FIG. **4**B is a cross-sectional view of a fourth embodiment of an analyte sensor, according to the invention;

FIGS. **5**A–**5**D is an expanded top view of a tip portion of the analyte sensor of FIG. **2**;

FIG. **6** is a cross-sectional view of a fifth embodiment of an analyte sensor, according to the invention;

FIG. **7** is an expanded top view of a tip-portion of the analyte sensor of FIG. **6**;

FIG. **8** is an expanded bottom view of a tip-portion of the analyte sensor of FIG. **6**;

FIG. **9** is a side view of the analyte sensor of FIG. **2**;

FIG. **10** is a top view of the analyte sensor of FIG. **6**;

FIG. **11** is a bottom view of the analyte sensor of FIG. **6**;

FIG. **12** is an expanded side view of one embodiment of a sensor and an insertion device, according to the invention;

FIGS. **13**A, **13**B, **13**C are cross-sectional views of three embodiments of the insertion device of FIG. **12**;

FIG. **14** is a cross-sectional view of one embodiment of a on-skin sensor control unit, according to the invention;

FIG. **15** is a top view of a base of the on-skin sensor control unit of FIG. **14**;

FIG. **16** is a bottom view of a cover of the on-skin sensor control unit of FIG. **14**;

FIG. **17** is a perspective view of the on-skin sensor control unit of FIG. **14** on the skin of a patient;

FIG. **18**A is a block diagram of one embodiment of an on-skin sensor control unit, according to the invention;

FIG. **18**B is a block diagram of another embodiment of an on-skin sensor control unit, according to the invention;

FIGS. **19**A, **19**B, **19**C, and **19**D are cross-sectional views of four embodiments of conductive contacts disposed on an interior surface of a housing of an on-skin sensor control unit, according to the invention;

FIGS. **19**E and **19**F are cross-sectional views of two embodiments of conductive contacts disposed on an exterior surface of a housing of an on-skin sensor control unit, according to the invention;

FIGS. **20**A and **20**B are schematic diagrams of two embodiments of a current-to-voltage converter for use in an analyte monitoring device, according to the invention;

FIG. **21** is a block diagram of one embodiment of an open loop modulation system for use in an analyte monitoring device, according to the invention;

FIG. **22** is a block diagram of one embodiment of a receiver/display unit, according to the invention;

FIG. **23** is a front view of one embodiment of a receiver/display unit;

FIG. **24** is a front view of a second embodiment of a receiver/display unit;

FIG. **25** is a block diagram of one embodiment of a drug delivery system, according to the invention;

FIG. **26** is a perspective view of the internal structure of an insertion gun, according to the invention;

FIG. **27**A is a top view of one embodiment of an on-skin sensor control unit, according to the invention;

FIG. **27**B is a top view of one embodiment of a mounting unit of the on-skin sensor control unit of FIG. **27**A;

FIG. **28**A is a top view of another embodiment of an on-skin sensor control unit after insertion of an insertion device and a sensor, according to the invention;

FIG. **28**B is a top view of one embodiment of a mounting unit of the on-skin sensor control unit of FIG. **28**A;

FIG. **28**C is a top view of one embodiment of a housing for at least a portion of the electronics of the on-skin sensor control unit of FIG. **28**A;

FIG. **28**D is a bottom view of the housing of FIG. **28**C; and

FIG. **28**E is a top view of the on-skin sensor control unit of FIG. **28**A with a cover of the housing removed.

While the invention is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is applicable to an analyte monitoring system using an implantable sensor for the in vivo determination of a concentration of an analyte, such as glucose or lactate, in a fluid. The sensor can be, for example, subcutaneously implanted in a patient for the continuous or periodic monitoring an analyte in a patient's interstitial fluid. This can then be used to infer the glucose level in the patient's bloodstream. Other in vivo analyte sensors can be made, according to the invention, for insertion into a vein, artery, or other portion of the body containing fluid. The analyte monitoring system is typically configured for monitoring the level of the analyte over a time period which may range from days to weeks or longer.

The following definitions are provided for terms used herein:

A "counter electrode" refers to an electrode paired with the working electrode, through which passes a current equal in magnitude and opposite in sign to the current passing through the working electrode. In the context of the invention, the term "counter electrode" is meant to include counter electrodes which also function as reference electrodes (i.e., a counter/reference electrode).

An "electrochemical sensor" is a device configured to detect the presence and/or measure the level of an analyte in a sample via electrochemical oxidation and reduction reactions on the sensor. These reactions are transduced to an electrical signal that can be correlated to an amount, concentration, or level of an analyte in the sample.

"Electrolysis" is the electrooxidation or electroreduction of a compound either directly at an electrode or via one or more electron transfer agents.

A compound is "immobilized" on a surface when it is entrapped on or chemically bound to the surface.

A "non-leachable" or "non-releasable" compound or a compound that is "non-leachably disposed" is meant to define a compound that is affixed on the sensor such that it does not substantially diffuse away from the working surface of the working electrode for the period in which the sensor is used (e.g., the period in which the sensor is implanted in a patient or measuring a sample).

Components are "immobilized" within a sensor, for example, when the components are covalently, ionically, or coordinatively bound to constituents of the sensor and/or are entrapped in a polymeric or sol-gel matrix or membrane which precludes mobility.

An "electron transfer agent" is a compound that carries electrons between the analyte and the working electrode, either directly, or in cooperation with other electron transfer agents. One example of an electron transfer agent is a redox mediator.

A "working electrode" is an electrode at which the analyte (or a second compound whose level depends on the level of the analyte) is electrooxidized or electroreduced with or without the agency of an electron transfer agent.

A "working surface" is that portion of the working electrode which is coated with or is accessible to the electron transfer agent and configured for exposure to an analyte-containing fluid.

A "sensing layer" is a component of the sensor which includes constituents that facilitate the electrolysis of the analyte. The sensing layer may include constituents such as an electron transfer agent, a catalyst which catalyzes a reaction of the analyte to produce a response at the electrode, or both. In some embodiments of the sensor, the sensing layer is non-leachably disposed in proximity to or on the working electrode.

A "non-corroding" conductive material includes non-metallic materials, such as carbon and conductive polymers.

Analyte Sensor Systems

The analyte monitoring systems of the present invention can be utilized under a variety of conditions. The particular configuration of a sensor and other units used in the analyte monitoring system may depend on the use for which the analyte monitoring system is intended and the conditions under which the analyte monitoring system will operate. One embodiment of the analyte monitoring system includes a sensor configured for implantation into a patient or user. For example, implantation of the sensor may be made in the arterial or venous systems for direct testing of analyte levels in blood. Alternatively, a sensor may be implanted in the interstitial tissue for determining the analyte level in interstitial fluid. This level may be correlated and/or converted to analyte levels in blood or other fluids. The site and depth of implantation may affect the particular shape, components, and configuration of the sensor. Subcutaneous implantation may be preferred, in some cases, to limit the depth of implantation of the sensor. Sensors may also be implanted in other regions of the body to determine analyte levels in other fluids. Examples of suitable sensor for use in the analyte monitoring systems of the invention are described in U.S. patent application, Ser. No. 09/034,372, incorporated herein by reference.

One embodiment of the analyte monitoring system **40** for use with an implantable sensor **42**, and particularly for use with a subcutaneously implantable sensor, is illustrated in block diagram form in FIG. **1**. The analyte monitoring system **40** includes, at minimum, a sensor **42**, a portion of which is configured for implantation (e.g., subcutaneous, venous, or arterial implantation) into a patient, and a sensor control unit **44**. The sensor **42** is coupled to the sensor control unit **44** which is typically attached to the skin of a patient. The sensor control unit **44** operates the sensor **42**, including, for example, providing a voltage across the electrodes of the sensor **42** and collecting signals from the sensor **42**. The sensor control unit **44** may evaluate the signals from the sensor **42** and/or transmit the signals to one or more optional receiver/display units **46**, **48** for evaluation. The sensor control unit **44** and/or the receiver/display units

46, 48 may display or otherwise communicate the current level of the analyte. Furthermore, the sensor control unit 44 and/or the receiver/display units 46, 48 may indicate to the patient, via, for example, an audible, visual, or other sensory-stimulating alarm, when the level of the analyte is at or near a threshold level. In some embodiments, a electrical shock can be delivered to the patient as a warning through one of the electrodes or the optional temperature probe of the sensor. For example, if glucose is monitored then an alarm may be used to alert the patient to a hypoglycemic or hyperglycemic glucose level and/or to impending hypoglycemia or hyperglycemia.

The Sensor

A sensor 42 includes at least one working electrode 58 formed on a substrate 50, as shown in FIG. 2. The sensor 42 may also include at least one counter electrode 60 (or counter/reference electrode) and/or at least one reference electrode 62 (see FIG. 8). The counter electrode 60 and/or reference electrode 62 may be formed on the substrate 50 or may be separate units. For example, the counter electrode and/or reference electrode may be formed on a second substrate which is also implanted in the patient or, for some embodiments of the implantable sensors, the counter electrode and/or reference electrode may be placed on the skin of the patient with the working electrode or electrodes being implanted into the patient. The use of an on-the-skin counter and/or reference electrode with an implantable working electrode is described in U.S. Pat. No. 5,593, 852, incorporated herein by reference.

The working electrode or electrodes 58 are formed using conductive traces 52 disposed on the substrate 50. The counter electrode 60 and/or reference electrode 62, as well as other optional portions of the sensor 42, such as a temperature probe 66 (see FIG. 8), may also be formed using conductive traces 52 disposed on the substrate 50. These conductive traces 52 may be formed over a smooth surface of the substrate 50 or within channels 54 formed by, for example, embossing, indenting or otherwise creating a depression in the substrate 50.

A sensing layer 64 (see FIGS. 3A and 3B) is often formed proximate to or on at least one of the working electrodes 58 to facilitate the electrochemical detection of the analyte and the determination of its level in the sample fluid, particularly if the analyte can not be electrolyzed at a desired rate and/or with a desired specificity on a bare electrode. The sensing layer 64 may include an electron transfer agent to transfer electrons directly or indirectly between the analyte and the working electrode 58. The sensing layer 64 may also contain a catalyst to catalyze a reaction of the analyte. The components of the sensing layer may be in a fluid or gel that is proximate to or in contact with the working electrode 58. Alternatively, the components of the sensing layer 64 may be disposed in a polymeric or sol-gel matrix that is proximate to or on the working electrode 58. Preferably, the components of the sensing layer 64 are non-leachably disposed within the sensor 42. More preferably, the components of the sensor 42 are immobilized within the sensor 42.

In addition to the electrodes 58, 60, 62 and the sensing layer 64, the sensor 42 may also include a temperature probe 66 (see FIGS. 6 and 8), a mass transport limiting layer 74 (see FIG. 9), a biocompatible layer 75 (see FIG. 9), and/or other optional components, as described below. Each of these items enhances the functioning of and/or results from the sensor 42, as discussed below.

The Substrate

The substrate 50 may be formed using a variety of non-conducting materials, including, for example, polymeric or plastic materials and ceramic materials. Suitable materials for a particular sensor 42 may be determined, at least in part, based on the desired use of the sensor 42 and properties of the materials.

In some embodiments, the substrate is flexible. For example, if the sensor 42 is configured for implantation into a patient, then the sensor 42 may be made flexible (although rigid sensors may also be used for implantable sensors) to reduce pain to the patient and damage to the tissue caused by the implantation of and/or the wearing of the sensor 42. A flexible substrate 50 often increases the patient's comfort and allows a wider range of activities. Suitable materials for a flexible substrate 50 include, for example, non-conducting plastic or polymeric materials and other non-conducting, flexible, deformable materials. Examples of useful plastic or polymeric materials include thermoplastics such as polycarbonates, polyesters (e.g., Mylar™ and polyethylene terephthalate (PET)), polyvinyl chloride (PVC), polyurethanes, polyethers, polyamides, polyimides, or copolymers of these thermoplastics, such as PETG (glycol-modified polyethylene terephthalate).

In other embodiments, the sensors 42 are made using a relatively rigid substrate 50 to, for example, provide structural support against bending or breaking. Examples of rigid materials that may be used as the substrate 50 include poorly conducting ceramics, such as aluminum oxide and silicon dioxide. One advantage of an implantable sensor 42 having a rigid substrate is that the sensor 42 may have a sharp point and/or a sharp edge to aid in implantation of a sensor 42 without an additional insertion device.

It will be appreciated that for many sensors 42 and sensor applications, both rigid and flexible sensors will operate adequately. The flexibility of the sensor 42 may also be controlled and varied along a continuum by changing, for example, the composition and/or thickness of the substrate 50.

In addition to considerations regarding flexibility, it is often desirable that implantable sensors 42 should have a substrate 50 which is non-toxic. Preferably, the substrate 50 is approved by one or more appropriate governmental agencies or private groups for in vivo use.

The sensor 42 may include optional features to facilitate insertion of an implantable sensor 42, as shown in FIG. 12. For example, the sensor 42 may be pointed at the tip 123 to ease insertion. In addition, the sensor 42 may include a barb 125 which assists in anchoring the sensor 42 within the tissue of the patient during operation of the sensor 42. However, the barb 125 is typically small enough that little damage is caused to the subcutaneous tissue when the sensor 42 is removed for replacement.

Although the substrate 50 in at least some embodiments has uniform dimensions along the entire length of the sensor 42, in other embodiments, the substrate 50 has a distal end 67 and a proximal end 65 with different widths 53, 55, respectively, as illustrated in FIG. 2. In these embodiments, the distal end 67 of the substrate 50 may have a relatively narrow width 53. For sensors 42 which are implantable into the subcutaneous tissue or another portion of a patient's body, the narrow width 53 of the distal end 67 of the substrate 50 may facilitate the implantation of the sensor 42. Often, the narrower the width of the sensor 42, the less pain the patient will feel during implantation of the sensor and afterwards.

For subcutaneously implantable sensors 42 which are designed for continuous or periodic monitoring of the analyte during normal activities of the patient, a distal end 67 of the sensor 42 which is to be implanted into the patient has

a width **53** of 2 mm or less, preferably 1 mm or less, and more preferably 0.5 mm or less. If the sensor **42** does not have regions of different widths, then the sensor **42** will typically have an overall width of, for example, 2 mm, 1.5 mm, 1 mm, 0.5 mm, 0.25 mm, or less. However, wider or narrower sensors may be used. In particular, wider implantable sensors may be used for insertion into veins or arteries or when the movement of the patient is limited, for example, when the patient is confined in bed or in a hospital.

Returning to FIG. **2**, the proximal end **65** of the sensor **42** may have a width **55** larger than the distal end **67** to facilitate the connection between contact pads **49** of the electrodes and contacts on a control unit. The wider the sensor **42** at this point, the larger the contact pads **49** can be made. This may reduce the precision needed to properly connect the sensor **42** to contacts on the control unit (e.g., sensor control unit **44** of FIG. **1**). However, the maximum width of the sensor **42** may be constrained so that the sensor **42** remains small for the convenience and comfort of the patient and/or to fit the desired size of the analyte monitor. For example, the proximal end **65** of a subcutaneously implantable sensor **42**, such as the sensor **42** illustrated in FIG. **1**, may have a width **55** ranging from 0.5 mm to 15 mm, preferably from 1 mm to 10 mm, and more preferably from 3 mm to 7 mm. However, wider or narrower sensors may be used in this and other in vivo applications.

The thickness of the substrate **50** may be determined by the mechanical properties of the substrate material (e.g., the strength, modulus, and/or flexibility of the material), the desired use of the sensor **42** including stresses on the substrate **50** arising from that use, as well as the depth of any channels or indentations formed in the substrate **50**, as discussed below. Typically, the substrate **50** of a subcutaneously implantable sensor **42** for continuous or periodic monitoring of the level of an analyte while the patient engages in normal activities has a thickness of 50 to 500 μm and preferably 100 to 300 μm. However, thicker and thinner substrates **50** may be used, particularly in other types of in vivo sensors **42**.

The length of the sensor **42** may have a wide range of values depending on a variety of factors. Factors which influence the length of an implantable sensor **42** may include the depth of implantation into the patient and the ability of the patient to manipulate a small flexible sensor **42** and make connections between the sensor **42** and the sensor control unit **44**. A subcutaneously implantable sensor **42** for the analyte monitor illustrated in FIG. **1** may have a length ranging from 0.3 to 5 cm, however, longer or shorter sensors may be used. The length of the narrow portion of the sensor **42** (e.g., the portion which is subcutaneously inserted into the patient), if the sensor **42** has narrow and wide portions, is typically about 0.25 to 2 cm in length. However, longer and shorter portions may be used. All or only a part of this narrow portion may be subcutaneously implanted into the patient. The lengths of other implantable sensors **42** will vary depending, at least in part, on the portion of the patient into which the sensor **42** is to be implanted or inserted.

Conductive Traces

At least one conductive trace **52** is formed on the substrate for use in constructing a working electrode **58**. In addition, other conductive traces **52** may be formed on the substrate **50** for use as electrodes (e.g., additional working electrodes, as well as counter, counter/reference, and/or reference electrodes) and other components, such as a temperature probe. The conductive traces **52** may extend most of the distance along a length **57** of the sensor **50**, as illustrated in FIG. **2**, although this is not necessary. The placement of the conductive traces **52** may depend on the particular configuration of the analyte monitoring system (e.g., the placement of control unit contacts and/or the sample chamber in relation to the sensor **42**). For implantable sensors, particularly subcutaneously implantable sensors, the conductive traces typically extend close to the tip of the sensor **42** to minimize the amount of the sensor that must be implanted.

The conductive traces **52** may be formed on the substrate **50** by a variety of techniques, including, for example, photolithography, screen printing, or other impact or non-impact printing techniques. The conductive traces **52** may also be formed by carbonizing conductive traces **52** in an organic (e.g., polymeric or plastic) substrate **50** using a laser. A description of some exemplary methods for forming the sensor **42** is provided in U.S. patent application Ser. No. 09/034,422, incorporated herein by reference.

Another method for disposing the conductive traces **52** on the substrate **50** includes the formation of recessed channels **54** in one or more surfaces of the substrate **50** and the subsequent filling of these recessed channels **54** with a conductive material **56**, as shown in FIG. **3A**. The recessed channels **54** may be formed by indenting, embossing, or otherwise creating a depression in the surface of the substrate **50**. Exemplary methods for forming channels and electrodes in a surface of a substrate can be found in U.S. patent application Ser. No. 09/034,422. The depth of the channels is typically related to the thickness of the substrate **50**. In one embodiment, the channels have depths in the range of about 12.5 to 75 μm (0.5 to 3 mils), and preferably about 25 to 50 μm (1 to 2 mils).

The conductive traces are typically formed using a conductive material **56** such as carbon (e.g., graphite), a conductive polymer, a metal or alloy (e.g., gold or gold alloy), or a metallic compound (e.g., ruthenium dioxide or titanium dioxide). The formation of films of carbon, conductive polymer, metal, alloy, or metallic compound are well-known and include, for example, chemical vapor deposition (CVD), physical vapor deposition, sputtering, reactive sputtering, printing, coating, and painting. The conductive material **56** which fills the channels **54** is often formed using a precursor material, such as a conductive ink or paste. In these embodiments, the conductive material **56** is deposited on the substrate **50** using methods such as coating, painting, or applying the material using a spreading instrument, such as a coating blade. Excess conductive material between the channels **54** is then removed by, for example, running a blade along the substrate surface.

In one embodiment, the conductive material **56** is a part of a precursor material, such as a conductive ink, obtainable, for example, from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). The conductive ink is typically applied as a semi-liquid or paste which contains particles of the carbon, metal, alloy, or metallic compound and a solvent or dispersant. After application of the conductive ink on the substrate **50** (e.g., in the channels **54**), the solvent or dispersant evaporates to leave behind a solid mass of conductive material **56**.

In addition to the particles of carbon, metal, alloy, or metallic compound, the conductive ink may also contain a binder. The binder may optionally be cured to further bind the conductive material **56** within the channel **54** and/or on the substrate **50**. Curing the binder increases the conductivity of the conductive material **56**. However, this is typically not necessary as the currents carried by the conductive material **56** within the conductive traces **52** are often rela-

tively low (usually less than 1 μA and often less than 100 nA). Typical binders include, for example, polyurethane resins, cellulose derivatives, elastomers, and highly fluorinated polymers. Examples of elastomers include silicones, polymeric dienes, and acrylonitrile-butadiene-styrene (ABS) resins. One example of a fluorinated polymer binder is Teflon® (DuPont, Wilmington, Del.). These binders are cured using, for example, heat or light, including ultraviolet (UV) light. The appropriate curing method typically depends on the particular binder which is used.

Often, when a liquid or semiliquid precursor of the conductive material **56** (e.g., a conductive ink) is deposited in the channel **54**, the precursor fills the channel **54**. However, when the solvent or dispersant evaporates, the conductive material **56** which remains may lose volume such that the conductive material **56** may or may not continue to fill the channel **54**. Preferred conductive materials **56** do not pull away from the substrate **50** as they lose volume, but rather decrease in height within the channel **54**. These conductive materials **56** typically adhere well to the substrate **50** and therefore do not pull away from the substrate **50** during evaporation of the solvent or dispersant. Other suitable conductive materials **56** either adhere to at least a portion of the substrate **50** and/or contain another additive, such as a binder, which adheres the conductive material **56** to the substrate **50**. Preferably, the conductive material **56** in the channels **54** is non-leachable, and more preferably immobilized on the substrate **50**. In some embodiments, the conductive material **56** may be formed by multiple applications of a liquid or semiliquid precursor interspersed with removal of the solvent or dispersant.

In another embodiment, the channels **54** are formed using a laser. The laser carbonizes the polymer or plastic material. The carbon formed in this process is used as the conductive material **56**. Additional conductive material **56**, such as a conductive carbon ink, may be used to supplement the carbon formed by the laser.

In a further embodiment, the conductive traces **52** are formed by pad printing techniques. For example, a film of conductive material is formed either as a continuous film or as a coating layer deposited on a carrier film. This film of conductive material is brought between a print head and the substrate **50**. A pattern on the surface of the substrate **50** is made using the print head according to a desired pattern of conductive traces **52**. The conductive material is transferred by pressure and/or heat from the film of conductive material to the substrate **50**. This technique often produces channels (e.g., depressions caused by the print head) in the substrate **50**. Alternatively, the conductive material is deposited on the surface of the substrate **50** without forming substantial depressions.

In other embodiments, the conductive traces **52** are formed by non-impact printing techniques. Such techniques include electrophotography and magnetography. In these processes, an image of the conductive traces **52** is electrically or magnetically formed on a drum. A laser or LED may be used to electrically form an image. A magnetic recording head may be used to magnetically form an image. A toner material (e.g., a conductive material, such as a conductive ink) is then attracted to portions of the drum according to the image. The toner material is then applied to the substrate by contact between the drum and the substrate. For example, the substrate may be rolled over the drum. The toner material may then be dried and/or a binder in the toner material may be cured to adhere the toner material to the substrate.

Another non-impact printing technique includes ejecting droplets of conductive material onto the substrate in a desired pattern. Examples of this technique include ink jet printing and piezo jet printing. An image is sent to the printer which then ejects the conductive material (e.g., a conductive ink) according to the pattern. The printer may provide a continuous stream of conductive material or the printer may eject the conductive material in discrete amounts at the desired points.

Yet another non-impact printing embodiment of forming the conductive traces includes an ionographic process. In the this process, a curable, liquid precursor, such as a photopolymerizable acrylic resin (e.g., Solimer 7501 from Cubital, Bad Kreuznach, Germany) is deposited over a surface of a substrate **50**. A photomask having a positive or negative image of the conductive traces **52** is then used to cure the liquid precursor. Light (e.g., visible or ultraviolet light) is directed through the photomask to cure the liquid precursor and form a solid layer over the substrate according to the image on the photomask. Uncured liquid precursor is removed leaving behind channels **54** in the solid layer. These channels **54** can then be filled with conductive material **56** to form conductive traces **52**.

Conductive traces **52** (and channels **54**, if used) can be formed with relatively narrow widths, for example, in the range of 25 to 250 μm, and including widths of, for example, 250 μm, 150 μm, 100 μm, 75 μm, 50 μm, 25 μm or less by the methods described above. In embodiments with two or more conductive traces **52** on the same side of the substrate **50**, the conductive traces **52** are separated by distances sufficient to prevent conduction between the conductive traces **52**. The edge-to-edge distance between the conductive traces is preferably in the range of 25 to 250 μm and may be, for example, 150 μm, 100 μm, 75 μm, 50 μm, or less. The density of the conductive traces **52** on the substrate **50** is preferably in the range of about 150 to 700 μm/trace and may be as small as 667 μm/trace or less, 333 μm/trace or less, or even 167 μm/trace or less.

The working electrode **58** and the counter electrode **60** (if a separate reference electrode is used) are often made using a conductive material **56**, such as carbon. Suitable carbon conductive inks are available from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). Typically, the working surface **51** of the working electrode **58** is at least a portion of the conductive trace **52** that is in contact with the analyte-containing fluid (e.g., implanted in the patient).

The reference electrode **62** and/or counter/reference electrode are typically formed using conductive material **56** that is a suitable reference material, for example silver/silver chloride or a non-leachable redox couple bound to a conductive material, for example, a carbon-bound redox couple. Suitable silver/silver chloride conductive inks are available from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). Silver/silver chloride electrodes illustrate a type of reference electrode that involves the reaction of a metal electrode with a constituent of the sample or body fluid, in this case, Cl^-.

Suitable redox couples for binding to the conductive material of the reference electrode include, for example, redox polymers (e.g., polymers having multiple redox centers.) It is preferred that the reference electrode surface be non-corroding so that an erroneous potential is not measured. Preferred conductive materials include less corrosive metals, such as gold and palladium. Most preferred

are non-corrosive materials including non-metallic conductors, such as carbon and conducting polymers. A redox polymer can be adsorbed on or covalently bound to the conductive material of the reference electrode, such as a carbon surface of a conductive trace **52**. Non-polymeric redox couples can be similarly bound to carbon or gold surfaces.

A variety of methods may be used to immobilize a redox polymer on an electrode surface. One method is adsorptive immobilization. This method is particularly useful for redox polymers with relatively high molecular weights. The molecular weight of a polymer may be increased, for example, by cross-linking.

Another method for immobilizing the redox polymer includes the functionalization of the electrode surface and then the chemical bonding, often covalently, of the redox polymer to the functional groups on the electrode surface. One example of this type of immobilization begins with a poly(4-vinylpyridine). The polymer's pyridine rings are, in part, complexed with a reducible/oxidizable species, such as $[Os(bpy)_2Cl]^{+/2+}$ where bpy is 2,2'-bipyridine. Part of the pyridine rings are quaternized by reaction with 2-bromoethylamine. The polymer is then crosslinked, for example, using a diepoxide, such as polyethylene glycol diglycidyl ether.

Carbon surfaces can be modified for attachment of a redox species or polymer, for example, by electroreduction of a diazonium salt. As an illustration, reduction of a diazonium salt formed upon diazotization of p-aminobenzoic acid modifies a carbon surface with phenylcarboxylic acid functional groups. These functional groups can then be activated by a carbodiimide, such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride. The activated functional groups are then bound with a amine-functionalized redox couple, such as the quaternized osmium-containing redox polymer described above or 2-aminoethylferrocene, to form the redox couple.

Similarly, gold can be functionalized by an amine, such as cystamine,. A redox couple such as $[Os(bpy)_2(pyridine\text{-}4\text{-}carboxylate)Cl]^{0/+}$ is activated by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride to form a reactive O-acylisourea which reacts with the gold-bound amine to form an amide.

In one embodiment, in addition to using the conductive traces **52** as electrodes or probe leads, two or more of the conductive traces **52** on the substrate **50** are used to give the patient a mild electrical shock when, for example, the analyte level exceeds a threshold level. This shock may act as a warning or alarm to the patient to initiate some action to restore the appropriate level of the analyte.

The mild electrical shock is produced by applying a potential between any two conductive traces **52** that are not otherwise connected by a conductive path. For example, two of the electrodes **58**, **60**, **62** or one electrode **58**, **60**, **62** and the temperature probe **66** may be used to provide the mild shock. Preferably, the working electrode **58** and the reference electrode **62** are not used for this purpose as this may cause some damage to the chemical components on or proximate to the particular electrode (e.g., the sensing layer on the working electrode or the redox couple on the reference electrode).

The current used to produce the mild shock is typically 0.1 to 1 mA. Higher or lower currents may be used, although care should be taken to avoid harm to the patient. The potential between the conductive traces is typically 1 to 10 volts. However, higher or lower voltages may be used depending, for example, on the resistance of the conductive traces **52**, the distance between the conductive traces **52** and the desired amount of current. When the mild shock is delivered, potentials at the working electrode **58** and across the temperature probe **66** may be removed to prevent harm to those components caused by unwanted conduction between the working electrode **58** (and/or temperature probe **66**, if used) and the conductive traces **52** which provide the mild shock.

Contact Pads

Typically, each of the conductive traces **52** includes a contact pad **49**. The contact pad **49** may simply be a portion of the conductive trace **52** that is indistinguishable from the rest of the trace **52** except that the contact pad **49** is brought into contact with the conductive contacts of a control unit (e.g., the sensor control unit **44** of FIG. **1**). More commonly, however, the contact pad **49** is a region of the conductive trace **52** that has a larger width than other regions of the trace **52** to facilitate a connection with the contacts on the control unit. By making the contact pads **49** relatively large as compared with the width of the conductive traces **52**, the need for precise registration between the contact pads **49** and the contacts on the control unit is less critical than with small contact pads.

The contact pads **49** are typically made using the same material as the conductive material **56** of the conductive traces **52**. However, this is not necessary. Although metal, alloys, and metallic compounds may be used to form the contact pads **49**, in some embodiments, it is desirable to make the contact pads **49** from a carbon or other non-metallic material, such as a conducting polymer. In contrast to metal or alloy contact pads, carbon and other non-metallic contact pads are not easily corroded if the contact pads **49** are in a wet, moist, or humid environment. Metals and alloys may corrode under these conditions, particularly if the contact pads **49** and contacts of the control unit are made using different metals or alloys. However, carbon and non-metallic contact pads **49** do not significantly corrode, even if the contacts of the control device are metal or alloy.

One embodiment of the invention includes a sensor **42** having contact pads **49** and a control unit **44** having conductive contacts (not shown). During operation of the sensor **42**, the contact pads **49** and conductive contacts are in contact with each other. In this embodiment, either the contact pads **49** or the conductive contacts are made using a non-corroding, conductive material. Such materials include, for example, carbon and conducting polymers. Preferred non-corroding materials include graphite and vitreous carbon. The opposing contact pad or conductive contact is made using carbon, a conducting polymer, a metal, such as gold, palladium, or platinum group metal, or a metallic compound, such as ruthenium dioxide. This configuration of contact pads and conductive contacts typically reduces corrosion. Preferably, when the sensor is placed in a 3 mM, and more preferably, in a 100 mM, NaCl solution, the signal arising due to the corrosion of the contact pads and/or conductive contacts is less than 3% of the signal generated by the sensor when exposed to concentration of analyte in the normal physiological range. For at least some subcutaneous glucose sensors, the current generated by analyte in a normal physiological range ranges from 3 to 500 nA.

Each of the electrodes **58**, **60**, **62**, as well as the two probe leads **68**, **70** of the temperature probe **66** (described below), are connected to contact pads **49** as shown in FIGS. **10** and **11**. In one embodiment (not shown), the contact pads **49** are on the same side of the substrate **50** as the respective electrodes or temperature probe leads to which the contact pads **49** are attached.

In other embodiments, the conductive traces **52** on at least one side are connected through vias in the substrate to contact pads **49***a* on the opposite surface of the substrate **50**, as shown in FIGS. **10** and **11**. An advantage of this configuration is that contact between the contacts on the control unit and each of the electrodes **58**, **60**, **62** and the probe leads **68**,**70** of the temperature probe **66** can be made from a single side of the substrate **50**.

In yet other embodiments (not shown), vias through the substrate are used to provide contact pads on both sides of the substrate **50** for each conductive trace **52**. The vias connecting the conductive traces **52** with the contact pads **49***a* can be formed by making holes through the substrate **50** at the appropriate points and then filling the holes with conductive material **56**.

Exemplary Electrode Configurations

A number of exemplary electrode configurations are described below, however, it will be understood that other configurations may also be used. In one embodiment, illustrated in FIG. **3**A, the sensor **42** includes two working electrodes **58***a*, **58***b* and one counter electrode **60**, which also functions as a reference electrode. In another embodiment, the sensor includes one working electrode **58***a*, one counter electrode **60**, and one reference electrode **62**, as shown in FIG. **3**B. Each of these embodiments is illustrated with all of the electrodes formed on the same side of the substrate **50**.

Alternatively, one or more of the electrodes may be formed on an opposing side of the substrate **50**. This may be convenient if the electrodes are formed using two different types of conductive material **56** (e.g., carbon and silver/silver chloride). Then, at least in some embodiments, only one type of conductive material **56** needs to be applied to each side of the substrate **50**, thereby reducing the number of steps in the manufacturing process and/or easing the registration constraints in the process. For example, if the working electrode **58** is formed using a carbon-based conductive material **56** and the reference or counter/reference electrode is formed using a silver/silver chloride conductive material **56**, then the working electrode and reference or counter/reference electrode may be formed on opposing sides of the substrate **50** for case of manufacture.

In another embodiment, two working electrodes **58** and one counter electrode **60** are formed on one side of the substrate **50** and one reference electrode **62** and a temperature probe **66** are formed on an opposing side of the substrate **50**, as illustrated in FIG. **6**. The opposing sides of the tip of this embodiment of the sensor **42** are illustrated in FIGS. **7** and **8**.

Sensing Layer

Some analytes, such as oxygen, can be directly electrooxidized or electroreduced on the working electrode **58**. Other analytes, such as glucose and lactate, require the presence of at least one electron transfer agent and/or at least one catalyst to facilitate the electrooxidation or electroreduction of the analyte. Catalysts may also be used for those analyte, such as oxygen, that can be directly electrooxidized or electroreduced on the working electrode **58**. For these analytes, each working electrode **58** has a sensing layer **64** formed proximate to or on a working surface of the working electrode **58**. Typically, the sensing layer **64** is formed near or on only a small portion of the working electrode **58**, often near a tip of the sensor **42**. This limits the amount of material needed to form the sensor **42** and places the sensing layer **64** in the best position for contact with the analyte-containing fluid (e.g., a body fluid, sample fluid, or carrier fluid).

The sensing layer **64** includes one or more components designed to facilitate the electrolysis of the analyte. The sensing layer **64** may include, for example, a catalyst to catalyze a reaction of the analyte and produce a response at the working electrode **58**, an electron transfer agent to indirectly or directly transfer electrons between the analyte and the working electrode **58**, or both.

The sensing layer **64** may be formed as a solid composition of the desired components (e.g., an electron transfer agent and/or a catalyst). These components are preferably non-leachable from the sensor **42** and more preferably are immobilized on the sensor **42**. For example, the components may be immobilized on a working electrode **58**. Alternatively, the components of the sensing layer **64** may be immobilized within or between one or more membranes or films disposed over the working electrode **58** or the components may be immobilized in a polymeric or sol-gel matrix. Examples of immobilized sensing layers are described in U.S. Pat. Nos. 5,262,035, 5,264,104, 5,264,105, 5,320,725, 5,593,852, and 5,665,222, U.S. patent application Ser. No. 08/540,789, and PCT Patent Application No. US98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998, incorporated herein by reference.

In some embodiments, one or more of the components of the sensing layer **64** may be solvated, dispersed, or suspended in a fluid within the sensing layer **64**, instead of forming a solid composition. The fluid may be provided with the sensor **42** or may be absorbed by the sensor **42** from the analyte-containing fluid. Preferably, the components which are solvated, dispersed, or suspended in this type of sensing layer **64** are non-leachable from the sensing layer. Non-leachability may be accomplished, for example, by providing barriers (e.g., the electrode, substrate, membranes, and/or films) around the sensing layer which prevent the leaching of the components of the sensing layer **64**. One example of such a barrier is a microporous membrane or film which allows diffusion of the analyte into the sensing layer **64** to make contact with the components of the sensing layer **64**, but reduces or eliminates the diffusion of the sensing layer components (e.g., a electron transfer agent and/or a catalyst) out of the sensing layer **64**.

A variety of different sensing layer configurations can be used. In one embodiment, the sensing layer **64** is deposited on the conductive material **56** of a working electrode **58***a*, as illustrated in FIGS. **3**A and **3**B. The sensing layer **64** may extend beyond the conductive material **56** of the working electrode **58***a*. In some cases, the sensing layer **64** may also extend over the counter electrode **60** or reference electrode **62** without degrading the performance of the glucose sensor. For those sensors **42** which utilize channels **54** within which the conductive material **56** is deposited, a portion of the sensing layer **64** may be formed within the channel **54** if the conductive material **56** does not fill the channel **54**.

A sensing layer **64** in direct contact with the working electrode **58***a* may contain an electron transfer agent to transfer electrons directly or indirectly between the analyte and the working electrode, as well as a catalyst to facilitate a reaction of the analyte. For example, a glucose, lactate, or oxygen electrode may be formed having a sensing layer which contains a catalyst, such as glucose oxidase, lactate oxidase, or laccase, respectively, and an electron transfer agent that facilitates the electrooxidation of the glucose, lactate, or oxygen, respectively.

In another embodiment, the sensing layer **64** is not deposited directly on the working electrode **58***a*. Instead, the sensing layer **64** is spaced apart from the working electrode **58***a*, as illustrated in FIG. **4**A, and separated from the working electrode **58***a* by a separation layer **61**. The sepa-

ration layer **61** typically includes one or more membranes or films. In addition to separating the working electrode **58***a* from the sensing layer **64**, the separation layer **61** may also act as a mass transport limiting layer or an interferent eliminating layer, as described below.

Typically, a sensing layer **64**, which is not in direct contact with the working electrode **58***a*, includes a catalyst that facilitates a reaction of the analyte. However, this sensing layer **64** typically does not include an electron transfer agent that transfers electrons directly from the working electrode **58***a* to the analyte, as the sensing layer **64** is spaced apart from the working electrode **58***a*. One example of this type of sensor is a glucose or lactate sensor which includes an enzyme (e.g., glucose oxidase or lactate oxidase, respectively) in the sensing layer **64**. The glucose or lactate reacts with a second compound (e.g., oxygen) in the presence of the enzyme. The second compound is then electrooxidized or electroreduced at the electrode. Changes in the signal at the electrode indicate changes in the level of the second compound in the fluid and are proportional to changes in glucose or lactate level and, thus, correlate to the analyte level.

In another embodiment, two sensing layers **63**, **64** are used, as shown in FIG. **4**B. Each of the two sensing layers **63**, **64** may be independently formed on the working electrode **58***a* or in proximity to the working electrode **58***a*. One sensing layer **64** is typically, although not necessarily, spaced apart from the working electrode **58***a*. For example, this sensing layer **64** may include a catalyst which catalyzes a reaction of the analyte to form a product compound. The product compound is then electrolyzed in the second sensing layer **63** which may include an electron transfer agent to transfer electrons between the working electrode **58***a* and the product compound and/or a second catalyst to catalyze a reaction of the product compound to generate a signal at the working electrode **58***a*.

For example, a glucose or lactate sensor may include a first sensing layer **64** which is spaced apart from the working electrode and contains an enzyme, for example, glucose oxidase or lactate oxidase. The reaction of glucose or lactate in the presence of the appropriate enzyme forms hydrogen peroxide. A second sensing layer **63** is provided directly on the working electrode **58***a* and contains a peroxidase enzyme and an electron transfer agent to generate a signal at the electrode in response to the hydrogen peroxide. The level of hydrogen peroxide indicated by the sensor then correlates to the level of glucose or lactate. Another sensor which operates similarly can be made using a single sensing layer with both the glucose or lactate oxidase and the peroxidase being deposited in the single sensing layer. Examples of such sensors are described in U.S. Pat. No. 5,593,852, U.S. patent application Ser. No. 08/540,789, and PCT Patent Application No. US98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998, incorporated herein by reference.

In some embodiments, one or more of the working electrodes **58***b* do not have a corresponding sensing layer **64**, as shown in FIGS. **3**A and **4**A, or have a sensing layer (not shown) which does not contain one or more components (e.g., an electron transfer agent or catalyst) needed to electrolyze the analyte. The signal generated at this working electrode **58***b* typically arises from interferents and other sources, such as ions, in the fluid, and not in response to the analyte (because the analyte is not electrooxidized or electroreduced). Thus, the signal at this working electrode **58***b* corresponds to a background signal. The background signal can be removed from the analyte signal obtained from other working electrodes **58***a* that are associated with fully-functional sensing layers **64** by, for example, subtracting the signal at working electrode **58***b* from the signal at working electrode **58***a*.

Sensors having multiple working electrodes **58***a* may also be used to obtain more precise results by averaging the signals or measurements generated at these working electrodes **58***a*. In addition, multiple readings at a single working electrode **58***a* or at multiple working electrodes may be averaged to obtain more precise data.

Electron Transfer Agent

In many embodiments, the sensing layer **64** contains one or more electron transfer agents in contact with the conductive material **56** of the working electrode **58**, as shown in FIGS. **3**A and **3**B. In some embodiments of the invention, there is little or no leaching of the electron transfer agent away from the working electrode **58** during the period in which the sensor **42** is implanted in the patient. A diffusing or leachable (i.e., releasable) electron transfer agent often diffuses into the analyte-containing fluid, thereby reducing the effectiveness of the electrode by reducing the sensitivity of the sensor over time. In addition, a diffusing or leaching electron transfer agent in an implantable sensor **42** may also cause damage to the patient. In these embodiments, preferably, at least 90%, more preferably, at least 95%, and, most preferably, at least 99%, of the electron transfer agent remains disposed on the sensor after immersion in the analyte-containing fluid for 24 hours, and, more preferably, for 72 hours. In particular, for an implantable sensor, preferably, at least 90%, more preferably, at least 95%, and most preferably, at least 99%, of the electron transfer agent remains disposed on the sensor after immersion in the body fluid at 37° C. for 24 hours, and, more preferably, for 72 hours.

In some embodiments of the invention, to prevent leaching, the electron transfer agents are bound or otherwise immobilized on the working electrode **58** or between or within one or more membranes or films disposed over the working electrode **58**. The electron transfer agent may be immobilized on the working electrode **58** using, for example, a polymeric or sol-gel immobilization technique. Alternatively, the electron transfer agent may be chemically (e.g., ionically, covalently, or coordinatively) bound to the working electrode **58**, either directly or indirectly through another molecule, such as a polymer, that is in turn bound to the working electrode **58**.

Application of the sensing layer **64** on a working electrode **58***a* is one method for creating a working surface for the working electrode **58***a*, as shown in FIGS. **3**A and **3**B. The electron transfer agent mediates the transfer of electrons to electrooxidize or electroreduce an analyte and thereby permits a current flow between the working electrode **58** and the counter electrode **60** via the analyte. The mediation of the electron transfer agent facilitates the electrochemical analysis of analytes which are not suited for direct electrochemical reaction on an electrode.

In general, the preferred electron transfer agents are electroreducible and electrooxidizable ions or molecules having redox potentials that are a few hundred millivolts above or below the redox potential of the standard calomel electrode (SCE). Preferably, the electron transfer agents are not more reducing than about −150 mV and not more oxidizing than about +400 mV versus SCE.

The electron transfer agent may be organic, organometallic, or inorganic. Examples of organic redox species are quinones and species that in their oxidized state have quinoid structures, such as Nile blue and indophenol.

Some quinones and partially oxidized quinhydrones react with functional groups of proteins such as the thiol groups of cysteine, the amine groups of lysine and arginine, and the phenolic groups of tyrosine which may render those redox species unsuitable for some of the sensors of the present invention because of the presence of the interfering proteins in an analyte-containing fluid. Usually substituted quinones and molecules with quinoid structure are less reactive with proteins and are preferred. A preferred tetrasubstituted quinone usually has carbon atoms in positions 1, 2, 3, and 4.

In general, electron transfer agents suitable for use in the invention have structures or charges which prevent or substantially reduce the diffusional loss of the electron transfer agent during the period of time that the sample is being analyzed. The preferred electron transfer agents include a redox species bound to a polymer which can in turn be immobilized on the working electrode. The bond between the redox species and the polymer may be covalent, coordinative, or ionic. Useful electron transfer agents and methods for producing them are described in U.S. Pat. Nos. 5,264,104; 5,356,786; 5,262,035; and 5,320,725, incorporated herein by reference. Although any organic or organometallic redox species can be bound to a polymer and used as an electron transfer agent, the preferred redox species is a transition metal compound or complex. The preferred transition metal compounds or complexes include osmium, ruthenium, iron, and cobalt compounds or complexes. The most preferred are osmium compounds and complexes. It will be recognized that many of the redox species described below may also be used, typically without a polymeric component, as electron transfer agents in a carrier fluid or in a sensing layer of a sensor where leaching of the electron transfer agent is acceptable.

One type of non-releasable polymeric electron transfer agent contains a redox species covalently bound in a polymeric composition. An example of this type of mediator is poly(vinylferrocene).

Another type of non-releasable electron transfer agent contains an ionically-bound redox species. Typically, this type of mediator includes a charged polymer coupled to an oppositely charged redox species. Examples of this type of mediator include a negatively charged polymer such as Nafion® (DuPont) coupled to a positively charged redox species such as an osmium or ruthenium polypyridyl cation. Another example of an ionically-bound mediator is a positively charged polymer such as quaternized poly(4-vinyl pyridine) or poly(1-vinyl imidazole) coupled to a negatively charged redox species such as ferricyanide or ferrocyanide. The preferred ionically-bound redox species is a highly charged redox species bound within an oppositely charged redox polymer.

In another embodiment of the invention, suitable non-releasable electron transfer agents include a redox species coordinatively bound to a polymer. For example, the mediator may be formed by coordination of an osmium or cobalt 2,2'-bipyridyl complex to poly(1-vinyl imidazole) or poly (4-vinyl pyridine).

The preferred electron transfer agents are osmium transition metal complexes with one or more ligands, each ligand having a nitrogen-containing heterocycle such as 2,2'-bipyridine, 1,10-phenanthroline, or derivatives thereof. Furthermore, the preferred electron transfer agents also have one or more ligands covalently bound in a polymer, each ligand having at least one nitrogen-containing heterocycle, such as pyridine, imidazole, or derivatives thereof. These preferred electron transfer agents exchange electrons rapidly between each other and the working electrodes **58** so that the complex can be rapidly oxidized and reduced.

One example of a particularly useful electron transfer agent includes (a) a polymer or copolymer having pyridine or imidazole functional groups and (b) osmium cations complexed with two ligands, each ligand containing 2,2'-bipyridine, 1,10-phenanthroline, or derivatives thereof, the two ligands not necessarily being the same. Preferred derivatives of 2,2'-bipyridine for complexation with the osmium cation are 4,4'-dimethyl-2,2'-bipyridine and mono-, di-, and polyalkoxy-2,2'-bipyridines, such as 4,4'-dimethoxy-2,2'-bipyridine. Preferred derivatives of 1,10-phenanthroline for complexation with the osmium cation are 4,7-dimethyl-1,10-phenanthroline and mono, di-, and polyalkoxy-1,10-phenanthrolines, such as 4,7-dimethoxy-1,10-phenanthroline. Preferred polymers for complexation with the osmium cation include polymers and copolymers of poly(1-vinyl imidazole) (referred to as "PVI") and poly(4-vinyl pyridine) (referred to as "PVP"). Suitable copolymer substituents of poly(1-vinyl imidazole) include acrylonitrile, acrylamide, and substituted or quaternized N-vinyl imidazole. Most preferred are electron transfer agents with osmium complexed to a polymer or copolymer of poly(1-vinyl imidazole).

The preferred electron transfer agents have a redox potential ranging from −100 mV to about +150 mV versus the standard calomel electrode (SCE). Preferably, the potential of the electron transfer agent ranges from −100 mV to +150 mV and more preferably, the potential ranges from −50 mV to +50 mV. The most preferred electron transfer agents have osmium redox centers and a redox potential ranging from +50 mV to −150 mV versus SCE.

Catalyst

The sensing layer **64** may also include a catalyst which is capable of catalyzing a reaction of the analyte. The catalyst may also, in some embodiments, act as an electron transfer agent. One example of a suitable catalyst is an enzyme which catalyzes a reaction of the analyte. For example, a catalyst, such as a glucose oxidase, glucose dehydrogenase (e.g., pyrroloquinoline quinone glucose dehydrogenase (PQQ)), or oligosaccharide dehydrogenase, may be used when the analyte is glucose. A lactate oxidase or lactate dehydrogenase may be used when the analyte is lactate. Laccase may be used when the analyte is oxygen or when oxygen is generated or consumed in response to a reaction of the analyte.

Preferably, the catalyst is non-leachably disposed on the sensor, whether the catalyst is part of a solid sensing layer in the sensor or solvated in a fluid within the sensing layer. More preferably, the catalyst is immobilized within the sensor (e.g., on the electrode and/or within or between a membrane or film) to prevent unwanted leaching of the catalyst away from the working electrode **58** and into the patient. This may be accomplished, for example, by attaching the catalyst to a polymer, cross linking the catalyst with another electron transfer agent (which, as described above, can be polymeric), and/or providing one or more barrier membranes or films with pore sizes smaller than the catalyst.

As described above, a second catalyst may also be used. This second catalyst is often used to catalyze a reaction of a product compound resulting from the catalyzed reaction of the analyte. The second catalyst typically operates with an electron transfer agent to electrolyze the product compound to generate a signal at the working electrode. Alternatively, the second catalyst may be provided in an interferent-eliminating layer to catalyze reactions that remove interferents, as described below.

One embodiment of the invention is an electrochemical sensor in which the catalyst is mixed or dispersed in the

conductive material **56** which forms the conductive trace **52** of a working electrode **58**. This may be accomplished, for example, by mixing a catalyst, such as an enzyme, in a carbon ink and applying the mixture into a channel **54** on the surface of the substrate **50**. Preferably, the catalyst is immobilized in the channel **53** so that it can not leach away from the working electrode **58**. This may be accomplished, for example, by curing a binder in the carbon ink using a curing technique appropriate to the binder. Curing techniques include, for example, evaporation of a solvent or dispersant, exposure to ultraviolet light, or exposure to heat. Typically, the mixture is applied under conditions that do not substantially degrade the catalyst. For example, the catalyst may be an enzyme that is heat-sensitive. The enzyme and conductive material mixture should be applied and cured, preferably, without sustained periods of heating. The mixture may be cured using evaporation or UV curing techniques or by the exposure to heat that is sufficiently short that the catalyst is not substantially degraded.

Another consideration for in vivo analyte sensors is the thermostability of the catalyst. Many enzymes have only limited stability at biological temperatures. Thus, it may be necessary to use large amounts of the catalyst and/or use a catalyst that is thermostable at the necessary temperature (e.g., 37° C. or higher for normal body temperature). A thermostable catalyst may be defined as a catalyst which loses less than 5% of its activity when held at 37° C. for at least one hour, preferably, at least one day, and more preferably at least three days. One example of a thermostable catalyst is soybean peroxidase. This particular thermostable catalyst may be used in a glucose or lactate sensor when combined either in the same or separate sensing layers with glucose or lactate oxidase or dehydrogenase. A further description of thermostable catalysts and their use in electrochemical inventions is found in U.S. Pat. No. 5,665,222 U.S. patent application Ser. No. 08/540,789, and PCT Application No. US98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998.

Electrolysis of the Analyte

To electrolyze the analyte, a potential (versus a reference potential) is applied across the working and counter electrodes **58**, **60**. The minimum magnitude of the applied potential is often dependent on the particular electron transfer agent, analyte (if the analyte is directly electrolyzed at the electrode), or second compound (if a second compound, such as oxygen or hydrogen peroxide, whose level is dependent on the analyte level, is directly electrolyzed at the electrode). The applied potential usually equals or is more oxidizing or reducing, depending on the desired electrochemical reaction, than the redox potential of the electron transfer agent, analyte, or second compound, whichever is directly electrolyzed at the electrode. The potential at the working electrode is typically large enough to drive the electrochemical reaction to or near completion.

The magnitude of the potential may optionally be limited to prevent significant (as determined by the current generated in response to the analyte) electrochemical reaction of interferents, such as urate, ascorbate, and acetaminophen. The limitation of the potential may be obviated if these interferents have been removed in another way, such as by providing an interferent-limiting barrier, as described below, or by including a working electrode **58***b* (see FIG. **3**A) from which a background signal may be obtained.

When a potential is applied between the working electrode **58** and the counter electrode **60**, an electrical current will flow. The current is a result of the electrolysis of the analyte or a second compound whose level is affected by the analyte. In one embodiment, the electrochemical reaction occurs via an electron transfer agent and the optional catalyst. Many analytes B are oxidized (or reduced) to products C by an electron transfer agent species A in the presence of an appropriate catalyst (e.g., an enzyme). The electron transfer agent A is then oxidized (or reduced) at the electrode. Electrons are collected by (or removed from) the electrode and the resulting current is measured. This process is illustrated by reaction equations (1) and (2) (similar equations may be written for the reduction of the analyte B by a redox mediator A in the presence of a catalyst):

$$nA(ox) + B \xrightarrow{\text{catalyst}} nA(red) + C \quad (1)$$

$$nA(red) \xrightarrow{\text{electrode}} nA(ox) + ne^- \quad (2)$$

As an example, an electrochemical sensor may be based on the reaction of a glucose molecule with two non-leachable ferricyanide anions in the presence of glucose oxidase to produce two non-leachable ferrocyanide anions, two hydrogen ions, and gluconolactone. The amount of glucose present is assayed by electrooxidizing the non-leachable ferrocyanide anions to non-leachable ferricyanide anions and measuring the current.

In another embodiment, a second compound whose level is affected by the analyte is electrolyzed at the working electrode. In some cases, the analyte D and the second compound, in this case, a reactant compound E, such as oxygen, react in the presence of the catalyst, as shown in reaction equation (3).

$$D + E \xrightarrow{\text{catalyst}} F + G \quad (3)$$

The reactant compound E is then directly oxidized (or reduced) at the working electrode, as shown in reaction equation (4)

$$nE(red) \xrightarrow{\text{electrode}} nE(ox) + ne^- \quad (4)$$

Alternatively, the reactant compound E is indirectly oxidized (or reduced) using an electron transfer agent H (optionally in the presence of a catalyst), that is subsequently reduced or oxidized at the electrode, as shown in reaction equations (5) and (6).

$$nH(ox) + E \rightarrow nH(red) + I \quad (5)$$

$$nH(red) \xrightarrow{\text{electrode}} nH(ox) + ne^- \quad (6)$$

In either case, changes in the concentration of the reactant compound, as indicated by the signal at the working electrode, correspond inversely to changes in the analyte (i.e., as the level of analyte increase then the level of reactant compound and the signal at the electrode decreases.)

In other embodiments, the relevant second compound is a product compound F, as shown in reaction equation (3). The product compound F is formed by the catalyzed reaction of analyte D and then be directly electrolyzed at the electrode or indirectly electrolyzed using an electron transfer agent and, optionally, a catalyst. In these embodiments, the signal arising from the direct or indirect electrolysis of the product compound F at the working electrode corresponds directly to the level of the analyte (unless there are other sources of the

product compound). As the level of analyte increases, the level of the product compound and signal at the working electrode increases.

Those skilled in the art will recognize that there are many different reactions that will achieve the same result; namely the electrolysis of an analyte or a compound whose level depends on the level of the analyte. Reaction equations (1) through (6) illustrate non-limiting examples of such reactions.

Temperature Probe

A variety of optional items may be included in the sensor. One optional item is a temperature probe **66** (FIGS. **8** and **11**). The temperature probe **66** may be made using a variety of known designs and materials. One exemplary temperature probe **66** is formed using two probe leads **68**, **70** connected to each other through a temperature-dependent element **72** that is formed using a material with a temperature-dependent characteristic. An example of a suitable temperature-dependent characteristic is the resistance of the temperature-dependent element **72**.

The two probe leads **68**, **70** are typically formed using a metal, an alloy, a semimetal, such as graphite, a degenerate or highly doped semiconductor, or a small-band gap semiconductor. Examples of suitable materials include gold, silver, ruthenium oxide, titanium nitride, titanium dioxide, indium doped tin oxide, tin doped indium oxide, or graphite. The temperature-dependent element **72** is typically made using a fine trace (e.g., a conductive trace that has a smaller cross-section than that of the probe leads **68**, **70**) of the same conductive material as the probe leads, or another material such as a carbon ink, a carbon fiber, or platinum, which has a temperature-dependent characteristic, such as resistance, that provides a temperature-dependent signal when a voltage source is attached to the two probe leads **68**, **70** of the temperature probe **66**. The temperature-dependent characteristic of the temperature-dependent element **72** may either increase or decrease with temperature. Preferably, the temperature dependence of the characteristic of the temperature-dependent element **72** is approximately linear with temperature over the expected range of biological temperatures (about 25 to 45° C.), although this is not required.

Typically, a signal (e.g., a current) having an amplitude or other property that is a function of the temperature can be obtained by providing a potential across the two probe leads **68**, **70** of the temperature probe **66**. As the temperature changes, the temperature-dependent characteristic of the temperature-dependent element **72** increases or decreases with a corresponding change in the signal amplitude. The signal from the temperature probe **66** (e.g., the amount of current flowing through the probe) may be combined with the signal obtained from the working electrode **58** by, for example, scaling the temperature probe signal and then adding or subtracting the scaled temperature probe signal from the signal at the working electrode **58**. In this manner, the temperature probe **66** can provide a temperature adjustment for the output from the working electrode **58** to offset the temperature dependence of the working electrode **58**.

One embodiment of the temperature probe includes probe leads **68**, **70** formed as two spaced-apart channels with a temperature-dependent element **72** formed as a cross-channel connecting the two spaced-apart channels, as illustrated in FIG. **8**. The two spaced-apart channels contain a conductive material, such as a metal, alloy, semimetal, degenerate semiconductor, or metallic compound. The cross-channel may contain the same material (provided the cross-channel has a smaller cross-section than the two spaced-apart channels) as the probe leads **68**, **70**. In other embodiments, the material in the cross-channel is different than the material of the probe leads **68**, **70**.

One exemplary method for forming this particular temperature probe includes forming the two spaced-apart channels and then filling them with the metallic or alloyed conductive material. Next, the cross-channel is formed and then filled with the desired material. The material in the cross-channel overlaps with the conductive material in each of the two spaced-apart channels to form an electrical connection.

For proper operation of the temperature probe **66**, the temperature-dependent element **72** of the temperature probe **66** can not be shorted by conductive material formed between the two probe leads **68**, **70**. In addition, to prevent conduction between the two probe leads **68**, **70** by ionic species within the body or sample fluid, a covering may be provided over the temperature-dependent element **72**, and preferably over the portion of the probe leads **68**, **70** that is implanted in the patient. The covering may be, for example, a non-conducting film disposed over the temperature-dependent element **72** and probe leads **68**, **70** to prevent the ionic conduction. Suitable non-conducting films include, for example, Kapton™ polyimide films (DuPont, Wilmington, Del.).

Another method for eliminating or reducing conduction by ionic species in the body or sample fluid is to use an ac voltage source connected to the probe leads **68**, **70**. In this way, the positive and negative ionic species are alternately attracted and repelled during each half cycle of the ac voltage. This results in no net attraction of the ions in the body or sample fluid to the temperature probe **66**. The maximum amplitude of the ac current through the temperature-dependent element **72** may then be used to correct the measurements from the working electrodes **58**.

The temperature probe can be placed on the same substrate as the electrodes. Alternatively, a temperature probe may be placed on a separate substrate. In addition, the temperature probe may be used by itself or in conjunction with other devices.

Another embodiment of a temperature probe utilizes the temperature dependence of the conductivity of a solution (e.g., blood or interstitial fluid). Typically, the conductivity of an electrolyte-containing solution is dependent on the temperature of the solution, assuming that the concentration of electrolytes is relatively constant. Blood, interstitial fluid, and other bodily fluids are solutions with relatively constant levels of electrolytes. Thus, a sensor **42** can include two or more conductive traces (not shown) which are spaced apart by a known distance. A portion of these conductive traces is exposed to the solution and the conductivity between the exposed portions of the conductive traces is measured using known techniques (e.g., application of a constant or known current or potential and measurement of the resulting potential or current, respectively, to determine the conductivity).

A change in conductivity is related to a change in temperature. This relation can be modeled using linear, quadratic, exponential, or other relations. The parameters for this relationship typically do not vary significantly between most people. The calibration for the temperature probe can be determined by a variety of methods, including, for example, calibration of each sensor **42** using an independent method of determining temperature (e.g., a thermometer, an optical or electrical temperature detector, or the temperature probe **66**, described above) or calibrating one sensor **42** and using that calibration for all other sensors in a batch based on uniformity in geometry.